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ZOOLOGY

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EDITOR

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ERRATA.

P. 251, line 31: *For* fuchsin-orange G-anilin blue, *read* fuchsin-orange G-anilin blue.

P. 259, under Zalesky 1866: *For* Bd. J 1, *read* Bd. 1; *for* Saeyler, *read* Seyler.

P. 264, under description of fig. 16: *For* ×1650, *read* ×825.

P. 266, under description of figs. 21, etc.: *For* ×1850, *read* ×925.

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ZOOLOGY

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A CASE OF PHYSIOLOGICAL POLARIZATION IN THE ASCIDIAN HEART

BY

FRANK W. BANCROFT AND C. O. ESTERLY

INTRODUCTION.

It is well known that, while in most animals the heart beats continuously in one direction only, in the Ascidians its contractions normally reverse their direction at fairly regular intervals. The earlier investigators, who studied the heart reversal chiefly in the intact animal, mostly concluded either that the cause of the reversal is the "necessity of the distribution of the arterial blood to all the organs" (Roule, 1884, p. 151), or that it is the increasing pressure that the heart has to labor against in forcing the blood through vessels that cannot easily accommodate it (Lahille, 1890, p. 292; Ritter, 1893, p. 75).

Recently the problem of the reversal has been attacked from a physiological point of view by Lingle (Loeb, 1900, pp. 28–29). Professor Loeb has informed us orally that the species used in these investigations was *Molgula* (*Bostrichiobranchus*) *manhattensis*. Lingle found that if the heart be divided at the center each half beats continuously from the end towards the cut; and also that the automatic activity was confined to two small regions near the ends, so that after these had been cut away they continued beating, while the long part between them no longer contracted in sea water. Professor Loeb has told us that the central piece did not contract in a solution of pure sodium

chloride. In commenting upon these results Loeb (1900, p. 29) says that they prove that the reversal is "determined by each of the two ends getting the upper hand alternately, and forcing the other to act in its rhythm for a while." This explanation was tested by the members of Professor Loeb's class in physiology at Wood's Hole, who found that towards the end of a series of contractions passing from one end, *a*, the beats become slower, or stop altogether. During this pause the other end, *b*, "succeeds in sending out a wave of contraction which reaches *a* before it has a chance to send out a wave of its own." Occasionally both ends contract at the same time, but the one which is about to stop delays in sending out its next contraction, and thus the beat from the end just beginning to contract can traverse the whole heart. Schultze (1901) in his study of the heart of Salpa came to the same conclusion as Loeb concerning the cause of the reversal, and confirmed most of the results of Lingle and Loeb's students. He found, in addition, that even when one end of the heart had been cut away, the rest of it which continued beating in one direction, regularly gave rise to alternating series of slower and faster contractions.* The slow series, he thought, corresponded to the time when, in the intact heart, the contractions would have been coming from the end which had been removed. He also discovered that a constant direction of contraction could be maintained by electrical stimulation of either end of the heart. This stimulus so increased the rate of contraction that the unstimulated end could not get control of the heart. In both Salpa and Ciona Schultze found that isolated pieces from the center of the heart would contract in sea water if they were left there long enough.

Neither Schultze nor any of the previous investigators of the subject have found ganglion cells in the Tunicate heart. Hunter (1902), however, has found in *Molgula manhattensis* a small collection of ganglion cells at both ends of the heart, where the contractions originate, and in a later paper (1903) has given

*We observed the same phenomenon in Ciona hearts from which one end had been removed. In such hearts series of normal and much slower contractions alternated. In some cases the heart would contract normally for a while, then stop entirely for a time that about corresponded to the duration of the series of slow contractons, and then beat normally again.

evidence for the conclusion that these ganglia are connected with the brain.

EXPERIMENTS.

The experiments here described were carried on at the San Pedro laboratory of the University of California during the summers of 1901 and 1902. They were almost entirely the work of the junior author, and were concerned, almost exclusively, especially in the second summer's work, with the problem of the physiological polarization of the Ciona heart, and not with the general question of the Tunicate heart-beat. For very kindly assistance and advice in connection with preparing the results for publication we wish to express our thanks to Professor Loeb.

Ciona intestinalis was the only species used; the heart being examined in sea water unless the contrary is stated. Pieces of the heart were separated from one another principally by cutting. When tied they gave in general the same results; but although no case of contractions passing a ligature was observed, isolation by tying was avoided on account of the possibility of such a passage.

Ordinarily (in 148 out 253 pieces) we found that pieces of the heart not connected with either end contracted spontaneously, and frequently these contractions began immediately after isolation. In other cases it was found that they began only after a variable quiescent interval. When pieces isolated in this way failed to contract automatically, contractions could almost invariably be started by immersion in a one per cent. sodium chloride solution. Equimolecular solutions of potassium chloride and calcium chloride had no such effect. Comparing these results with those of Lingle and Schultze on the automaticity of pieces of the heart, it will be seen that different species, and even the same species at different places, may differ in this respect. This difference need not surprise us when we remember the variability of living beings in general. It may be due to the presence of ganglion cells in the central part of the heart in some species, or to some other characteristic of the tissues which would make them less sensitive to the action of the constituents of the sea water that tend to inhibit automatic contractions.

We have seen that Lingle and Schultze agree that if a part of a tunicate heart be physiologically connected with but one of its ends, the contractions continue uninterruptedly from that end. This result we obtained in most cases, though occasional exceptions were encountered. Now the fact that we wish particularly to emphasize, and to the consideration of which this paper is devoted, is that *not only does the direction of the contractions remain fixed while a part of the heart is connected with only one of its ends,* but that in some way a change is effected in the heart tissue so that the direction of the contractions still remains fixed after the part has been isolated from the end which was instrumental in producing the fixation.* That is, we may say that the heart tissue has become physiologically polarized by being left in contact for a while with only one end of the heart.

Experiments directed merely to determining whether such a polarization is a fact consisted first in leaving a part of the heart connected with only one of the ends for a while. It was then isolated from this end, the direction of the contractions noted, and finally frequently divided into still smaller pieces, and the direction of the contraction in each recorded. The character of the evidence is best made clear by reference to a typical experiment:

Experiment 39a.—

10:37—Visceral† end of heart removed. The contractions are ab-branchial.†

10:44—Pulsations have continued in the same direction. Branchial† end removed. Pulsations in the long central loop of the heart are still ab-branchial.

10:54—Visceral side of the loop cut in half.

10:58—Pulsations in both the pieces thus formed are ab-branchial.

*It should be stated that in all of these experiments the two ends behaved the same. We could not see that it made any difference which end was cut away first.

†In Ciona one end, which is attached to the viscera, is called the visceral, and the other, which connects with the branchial sac, is called the branchial end. Contractions passing in the direction from the branchial towards the visceral end are called ab-branchial, those passing in the opposite direction ab-visceral. This nomenclature is that of Schultze slightly modified.

11:07—Cut branchial side loop in half. Small central loop is contracting, but the direction cannot be made out. The other two pieces are contracting ab-branchially.

11:14—All three pieces are now clearly contracting in the original ab-branchial direction.

1:46—All contractions have stopped.

In some cases the pieces failed to contract and immersion in the sodium chloride solution was necessary before the direction of the heart-beat could be recorded. Contractions were obtained from isolated pieces of 51 hearts experimented upon in this way; and in 41 (or 80 per cent.) of them all the isolated pieces that contracted at all did so in the direction they had before being isolated. From some of these hearts as many as four or five pieces all contracting in the fixed direction* and unconnected with either end were obtained. From the ten hearts that did not behave normally many isolated pieces that contracted in the direction of fixation were also obtained. But as in all of these hearts at least one piece did not follow the normal law they were considered as furnishing evidence against polarization. However, in spite of this method of estimating the evidence which is decidedly unfavorable to our theory, still the preponderance of evidence in favor of polarization is too large to have been due to accident.

It seemed possible, however, that the persistence of the fixed direction after isolation of the pieces might not be due to a change in the heart tissues, but that the result might have been caused by a tendency for the pieces always to beat in the direction *away* from the most recent cut, which could then be considered as the stimulus controlling the direction of the contractions.

The most convincing type of experiment bearing on this question consisted in first removing one end of the heart to allow the direction of the contractions to become fixed. Then the second end was also removed, and a long loop connected with either end and pulsating in the direction of fixation was obtained. Five of six small pieces were now cut from the end of the loop

*By contraction in the fixed direction or the direction of fixation we mean the direction of the contractions when the part of the heart in question was connected with only one of the ends of the heart.

toward which the contractions were travelling; but the result of these experiments was always negative. The cuts did not change the direction of the contractions. Finally, to make the consideration of the evidence complete, all the cuts made were considered from this point of view, and it was found that in the great majority of cases the direction of the contractions did not change after the cut. In a few cases it did change, but the character of the change was not a constant one, for after the change the direction of the contraction was *toward* the most recent cut, exactly as frequently as it was away from it. *It is very clear then, that the stimulus due to isolating the pieces cannot account for the persistence of the fixed direction of contraction.*

Another possible explanation of the phenomenon is that it may be caused by the refractory qualities of the Ciona heart during and immediately following each contraction. Schultze (1901) found that the Salpa heart has a refractory period, similar to that found in Vertebrates, during which no stimulus could provoke an extra contraction. Now if a piece of beating Ciona heart be isolated, one end of it will will have finished contracting before the other, and might consequently be in a better position to originate spontaneous contractions than the other. The contractions would consequently start at the end of the isolated piece which formerly contracted first. That is, they would continue in their original direction.

This possibility was tested in two ways:

In the first place, if this explanation is correct, then the direction of contractions *immediately* preceding isolation is the important thing, and it should not matter whether the piece was isolated during a normal series, or after the direction of contractions had been rigidly fixed by first removing one end. Accordingly pieces of the heart were isolated during the normal series, and it was found that in 13 hearts out of 21 (or 62 per cent.) the pieces did not change their direction after isolation. These results seem to indicate that there is a tendency for pieces of the heart isolated during the normal series to maintain the original direction of contraction after isolation; but this tendency is much weaker than in those cases where the direction was fixed before isolation.

Secondly, if the direction, in which an isolated piece of the heart beats, depends upon the fact that previous to isolation one end had contracted before the other and consequently had recovered its excitability more completely; then, if the heart could be made to stop beating for a short time, the excitability of the two ends would soon become equalized, and there should be no relation between the direction of contraction before and after isolation. Accordingly, to test this explanation, our records of experiments in which pieces stopped after isolation and then started again when brought into the sodium chloride solution was gone over. They showed that after a quiescent period of from 3 to 90 minutes, 13 of the 14 pieces experimented upon contracted in the direction they had before immersion in the salt solution. Since the quiescent interval in these experiments were very much longer than the normal interval between contractions, the results, in spite of their small number, make it very improbable that the differential recovery of the two ends of the pieces from the lowered excitability following each contraction is the cause of the fixed direction of the contractions in isolated pieces of the Ciona heart.

As neither of the two possibilities considered accounts for the phenomena, we are forced to conclude that connection with only one end of the heart brings about a change of some kind in the tissues as the piece so connected continues after isolation to beat in the previous direction. This change may be termed a physiological polarization, but whether it is caused by the long continued constant direction of the contractions or the connection with one end only, apart from the direction of the contractions, we cannot say. So far as we know similar cases of polarization have not been described.

DEVIATIONS FROM NORMAL BEHAVIOR.

Occasionally the normal behavior of the heart, which presented strong evidence in favor of polarization, was replaced by contractions of the most varied kind, which will be briefly described.

Pulsations from both ends at the same time, which have been seen by both Loeb and Schultze, were observed in two of seven Cionas, in which puncturing the body wall had exposed the

heart without in any way injuring it. These contractions do not appear to have been ante-mortem phenomena like those observed by Schultze, but rather an exaggeration of the similar contractions described by Loeb at the time of reversal, for at first they alternated with the normal series of contractions, and were finally entirely replaced by the normal series.

Pulsations from the center of the heart were observed:

1. In 2 cases out of the 7 described above in which the heart was isolated without injury.

2. In two cases out of 63 in which one end of the heart had been isolated from the remainder.

3. In 8 out of 28 half hearts obtained by tying the heart across its center.

4. Occasionally in still smaller pieces.

In some of these cases the contractions came steadily from the center, while in others the direction was sometimes reversed.

Reversals in pieces of the heart subjected to no external influences, except the sea water in which they were immersed, were noted:

1. In 2 hearts from which only one end had been removed. Series of pulsations from the center alternated with series from the intact end.

2. In 3 of the 28 half hearts: Series from the center alternating with the series from the end.

3. In 4 out of 82 still smaller pieces one end of which was an intact end of the heart.

4. In 3 out of 158 pieces which were not connected with an end of the heart. In one of these, series from the center alternated with series from one of the cut ends, in the others the contractions began at the cut ends.

These observations on contractions from the center and the reversal of small pieces of the heart show that the heart of Ciona as obtained at San Pedro is of a more uniform character throughout its whole extent than had been formerly supposed. The tissues at the end are of such a character that ordinarily the contractions start there. But the center resembles them so closely that even in the intact heart contractions may originate in that place. In fact, all parts of the heart are so remarkably similar

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EMBRYOLOGY AND EMBRYONIC FISSION IN THE GENUS CRISIA.*

BY

ALICE ROBERTSON.

INTRODUCTION.

The processes of embryonic fission in the Cyclostomata were first made known a few years ago by Dr. Sidney F. Harmer. That investigator found that this unique process of reproduction of the embryo occurs in several somewhat distantly related genera of the subclass, viz., in *Crisia*, in *Lichenopora*, and in *Tubulipora*. The facts disclosed were so interesting and remarkable, that further study of the phenomena was deemed desirable, both for the corroboration of the results, and for the purpose of completing more of the details. The investigation reported in the following pages has been made upon *Crisia* only, several species of which occur abundantly in the vicinity of San Francisco Bay. The chief results of Dr. Harmer's investigations, that is, the discovery of the occurrence in this genus of a budding of the embryo, the separation of the buds from the mother embryo, and their ultimate transformation into free swimming larvæ, have been fully confirmed. Besides as thorough a study as possible has been made of the origin of the genital products, both male and female. Some unique features have been found in the origin and development of these elements, all of which may be interpreted as secondary modifications due to the high degree of colonial specialization to which these bryozoa have attained.

*Dissertation presented to the Faculty of the College of Natural Sciences of the University of California in partial fulfillment of the requirement for the Degree of Doctor of Philosophy.

Technique.—For this investigation, material has been collected each month, and twice in the month during the spring, when the tides were favorable. Although specimens have been secured from various localities, they have been regularly obtained from a locality known as Lands End, near the entrance of the Golden Gate, California. The results most relied upon have been obtained then, from material killed and fixed under the most favorable circumstances, *i.e.*, very soon after collection. The relatively thick calcareous ectocyst of *Crisia* makes it difficult to fix the tissues rapidly enough to prevent their shrinkage and consequent distortion. The most successful results were obtained by the use of a solution of hot corrosive sublimate. In most cases a solution of this with glacial acetic was used, in other cases, the hot corrosive sublimate alone. The specimens were allowed to remain in the fixing fluid only long enough to become penetrated, when they were washed in 50% alcohol containing iodine. After this, they were carried through the various grades of alcohol and finally preserved in 85% alcohol. The process of killing and fixing did not include decalcification. Such portions only as were required for mounting, were afterward completely decalcified. In the process of decalcification, much trouble is frequently experienced by the formation of bubbles of gas. It was found easy to avoid these, however, and the consequent tearing of the tissues, by decalcifying small pieces in a high grade of alcohol made weakly acid. The stains used were Delafield's and Ehrlich's hæmatoxylin with eosin; Benda's iron hæmatoxylin alone, and with eosin and fuchsin; and Auerbach's mixture of methyl green and fuchsin. Many other stains were experimented with, but these gave the most satisfactory results.

Four species of *Crisia* are more or less abundant in this region, viz., *Crisia eburnea*, *Crisia geniculata*, *Crisia cornuta*, and a new species, *Crisia occidentalis*. A full description of this last species will follow in a later paper. Special reference is made in this paper to *Crisia eburnea*, although all the species have been studied more or less in regard to their method of reproduction. *Crisia eburnea* is certainly dicœcious, the two kinds of genital products never being found in the same colony. This is thought to be true also of *Crisia occidentalis*, although the evi-

dence is less conclusive for this species. The other two, *Crisia geniculata* and *Crisia cornuta* are probably monœcious.

REPRODUCTIVE PROCESSES.

SEXUAL ELEMENTS.

1. *Origin of the Male Genital Products.—Crisia*, and perhaps other genera of the Cyclostomata, differ from the rest of the bryozoa in the production of the sexual elements. In young and growing colonies of this genus these products originate and are differentiated as such, *at the tips of the branches*. This can best be seen in the spring when the colonies are growing actively, and when the germinal tissue is in the healthiest condition. During the fall and winter months the tissue is thin even at the growing points, stains badly, and is in a degenerated state. In the latter part of February and throughout March, April, and May, however, both sorts of germinal cells are abundant, and form very conspicuous objects in all the young tips. The tissue at the growing points at this time forms a thick layer of "embryonic" cells closely packed together and staining deeply in hæmatoxylin. It is here differentiated into two layers which form the body wall, or lining of the zoœcia. Pl. XII, Fig. 1, represents the tip of a branch of *Crisia eburnea*, which has been decalcified, stained and mounted in toto. It consists of two series of zoœcia (z^1 and z^2) lying side by side. At the growing point (*gr. tis.*) the zoœcia are cut off alternately from the outer edges, the bases (*b.*) or proximal extremities of each pair being in contact, while their distal portions are separated by the bases of the next succeeding pair. The branch is thus somewhat flattened, having a dorsal (*d.*) and a ventral side (*v.*), and a right (*r.*) and a left (*l.*) edge. The growing point includes that portion which is anterior to the youngest pair of zoœcia and consists of two parts, (*a*) the layers of deeply staining cells (*gr. tis.*), and (*b*), the budding region. This latter is represented in Fig. 1 by young polypides (*pd. bd.*) in various stages of advancement. These portions are again shown in Pl. XIII, Fig. 18, which represents the tip of an actively growing branch containing, besides a developing ovicell (*ovl.*), a number of young polypides (*pd.*).

The cell layers which make up the body wall of a colony may be distinctly seen in section. Pl. XII, Fig. 2, represents a section from a growing tip of a male colony, in which the outer, or ectodermal layer consists of small rounded cells (*ec. cls.*), while the inner or mesodermal layer consists of much larger cells possessing a distinct large nucleus (*mes. cls.*). It is part of this inner layer which becomes modified into a germinal epithelium, (*ger. cls.*), and from which both ova and spermatozoa originate. Pl. XII, Fig. 3, is a section from the same series representing much the same characters. If these two sections be compared, the mesodermal cells in each (*mes. cls.*) are seen to be of various sizes. Many are of normal size (*mes. cls.*), while others are much larger, and constitute the cells of the male germinal epithelium (*ger. cls.*). In the germ cells, the nucleus and nucleolus have increased in size, and are surrounded by a layer of finely granular cytoplasm. The mesodermal cells which go to form the parietal layer are of various sizes and shapes, but of similar appearance. The ectodermal cells are either rounded or elongated, depending upon the portion of the tip in which they are. Near the edges, right and left, they are round, while near the middle they become much elongated and less numerous, (Pl. XII, Figs. 10 and 11, *ec. cls.*).

The relation between the polypide buds and the germinal tissue is shown in Fig. 4, a section from a male colony which represents several stages in the development of the polypides. At the anterior edge, in the angle toward the left (*l.*) the germinal cells may be seen (*ger. cls.*). Proximal to this point, a mass of cells represents the youngest polypide bud (*pd. bd. 2*), and below this there is an older bud (*pd. bd.* 1) in which the cavity of the stomach is formed (*st.*). As the distal portion of the branch continues to grow, the fully formed germinal cells are left behind at or near a point where a polypide bud forms, and in a male colony a few of these cells become attached to each bud constituting the testis of the developing polypides. In Pl. XII, Fig. 4, a number of large cells closely resembling the cells of the germinal tissue in size and appearance of the nucleus, are attached to the stomach of the older polypide bud (*pd. bd*

1., *tes.*). Below the stomach of the polypide (*pd.*) is a similar but larger mass of cells constituting the testis of that animal (*tes.*). If more of the branch of which Fig. 4 is a section could be shown, each succeeding polypide would be found to possess a corresponding structure. Examination of a series of polypides shows that the development of the testis proceeds with that of the polypide, the lower and hence the older polypides possessing the larger testis.

The spermatozoa, two of which are shown in Fig. 5, may be found clustered about large cells which are more or less abundant throughout the testis, or may be seen passing in a stream through the testis toward its distal portion, to a point at the base of the tentacles. Their actual egress was not detected, so that it is not known whether it occurs through a definite opening or only after the degeneration of the polypide, as is the case in most bryozoa. Harmer ('93) mentions the escape of the spermatoza of *Crisia cornuta* through the aperture of the zoœcium, but fails to state whether or not the polypide had degenerated. Hincks ('80) observed them passing in a stream through the intertentacular organ. The ectoprocts are not thought to have a sperm duct, the sperm escaping presumably through the orifice of a zoœcium after the polypide has degenerated. Since in most cases ova and spermatozoa are produced in the same zoœcium, either simultaneously or in succession, the necessity for a means of egress so that the one may reach the other is not so important. It is possible that in *Crisia* they may escape before the death of the polypide, and what evidence I have would indicate that those that mature do so while the polypide is still intact. In examining a quantity of material, however, the scarcity of ripe spermatozoa is very noticeable. In the spring, at least, the male genital products can be obtained in abundance and in various stages of development, but one searches almost in vain for spermatozoa. In a collection of preparations representing a hundred or more polypides, and made from material obtained during the season when the sexual elements are most abundant, in only *one instance* was ripe sperm found. Fig. 7 represents a section of living testis in a somewhat advanced stage of development, showing a typical arrangement and appearance of the

cells. These are in groups of darkly staining nuclei, sometimes arranged in large numbers around a central mass of cytoplasm, very frequently in groups of four nuclei imbedded in a mass of cytoplasm. (Pl. XII, Fig. 7, *tet*, and Fig. 7A.) The individual members of these tetrads consist sometimes of solid masses of chromatin, sometimes of an outer layer of chromatin surrounding a vacuole. Whether vacuolated or not, these probably represent stages in the development of spermatozoa—a development which apparently proceeds no further. Without making an exhaustive study of the spermatogenesis, it is, of course, impossible to state positively that degeneration of the testis occurs at this stage in the development of the sperm cells, and such a study has not been made; but in view of the evidence adduced, the suggestion that the testis does thus degenerate is worth consideration.

In examining branches of male colonies in which regeneration is taking place, the quantity of degenerated material in each zoœcium is unusually large as compared with that found in other bryozoa. Such a mass of material is shown in Fig. 6, which represents a section of a zoœcium containing a small regenerating polypide (*re. pd.*) and the remains of a degenerated polypide, the former occupant of the zoœcium (*b. b.*). In this "brown body" two portions can be distinguished, a round, somewhat homogeneous mass representing the tentacles and alimentary canal of the degenerated polypide (*de. pd.*), and a long, tapering mass extending almost to the base of the zoœcium representing, perhaps, the degenerated testis (*de. tes.*). This latter occupies the position of the testis and closely resembles it in appearance, both of the whole mass and of the individual groups of cells, among which the tetrads, both vacuolated and non-vacuolated, can be detected.

Comparing the early regenerating stages of male and female colonies, the quantity of material in the "brown bodies" in the latter is smaller than that in the male, and represents the degenerated polypides only. Each is at first a homogeneous mass which later disintegrates more or less, and falls into the base of the zoœcium. In the later stages, when the regenerated polypide has attained its full growth, the difference in appearance of the

"brown bodies" of the sexes is not so apparent. In both the residue becomes pushed into the extreme base of the zoœcium and is packed into smaller space.

The evidence for degeneration which is afforded by the scarcity of spermatozoa, and by the resemblance between the "brown body" of the male colonies and the testis, is strengthened by its probable correlation with what occurs in the female colonies. Here, as will be shown, large numbers of ova are produced, but on account of the reproduction peculiar to *Crisia*, relatively few give rise to larvæ, hence a relatively small number of sperm are functionally necessary. Degeneration of the male genital product, if it occur, is to be regarded, then, as a secondary modification correlated with the fact that every egg that contributes to the perpetuation of the species produces, through embryonic fission, not one, but a great many colonies.

2. *Origin of the Female Genital Products.*—In the female colonies of *Crisia eburnea* the ova arise as do the male germ cells, from the mesoderm of the growing tip of the branches. They are *differentiated at the tip of the branches, and in no other part of the colony.* Pl. XII, Fig. 8, represents a section from the ventral side of a female colony, in which the two layers of the body wall are distinctly shown. Close to the anterior edge is a row of small round ectodermal cells (*ec. cls.*), forming the outer layer, while inside of this is a layer of larger cells possessing very large distinct nuclei, and constituting the mesodermal layer (*mes. cls.*). The cells of this layer perform various rôles in the economy of the colony, some giving rise to part of the parietal lining of the zoœcia, some being transformed into the mesenchymatous tissue of the branches (*mes. tis.*), and the remainder producing the germinal epithelium. If a comparison be made between Fig. 8 of a female colony, and Fig. 2 and 3 of a male colony, no difference will be recognized in the cells of this tissue. In both, the germinal cells are of the same size, and bear identical relations to the growing points. It was shown for the male colonies that the germinal cells are more numerous in the angles, right and left, of the tip. This is true also of the female colonies, as may be seen in Pl. XII,

Figs. 9 and 10, which represent serial sections from the growing tip of a female colony, each of which shows the accumulations of modified mesodermal cells in the angles of the branch (*ger. cls.*). They are found, too, at a time earlier than that at which the polypide bud appears. This is especially clear in Pl. XII, Fig. 11, representing a section of the bud-forming region of a female colony. At the anterior edge of the tip are the germ cells (*ger. cls.*), while proximal to these is a series of polypide buds in various stages of development. In the oldest bud (*pd. bd. 1*) the cavity of the stomach is visible (*st.*). No ova have united with any of these buds, and an examination of older portions of the branch does not reveal their existence in the older zoœcia. On the other hand, numberless sections prove that not only are single, detached *ova* produced at the anterior extremities of the branch, but it is in these places that the *ovaries* are located. Evidence of this is given in Pl. XII, Fig. 12, and Pl. XIII, Figs. 13 and 14, consecutive sections taken somewhat obliquely through the germinal region of the tip of a colony. The line of cells (*sep.*) in the three sections, represents different parts of the same septum. Fig. 12, the first section of the series, is composed mainly of cells forming the ventral wall, the heap of cells lying near the septum (*pd. bd. 2*) representing the outer layer of a polypide bud. Fig. 13 represents the same polypide bud (*pd. bd. 2*), while proximal to it is another (*pd. bd. 1*). Distal to the anterior bud (*pd. bd. 2*) in this section, five cells of an ovary are shown, one of which (*ov.*) has advanced considerably in development. Fig. 14 shows an ovum (*ov.*) from the same ovary, which lies in close proximity to a polypide bud (*pd. bd. 1*). From this point forward there extends to the tip of the branch, an almost unbroken line of ova, constituting an ovary. A similar condition is represented in Pl. XIII, Fig. 15, a section from another colony, where several ova lie close to the septum (*sep.*), within the cavity of the branch. These are in close proximity to a mass of small cells (*pd. bd. 2*), and constitute the older portion of an ovary (*ovy.*), which as succeeding sections show, extends forward to the anterior edge of the branch. Examination of a great number of series reveals the same condition, *i.e.*, the formation of groups of ova, or ovaries at the tips

of the branches. Such a precocious appearance of ova is reported in a few instances among the Cheilostomata (Calvet '00), but as far as I am aware no other case is known in which the *ovary itself* is thus precociously formed. The early appearance of the germ cells in *Crisia* is somewhat comparable to what takes place in the *Hydromedusae* ('90). In both classes of animals it is a secondary condition correlated with the subordination of the sexual individuals, and the assumption by the colony of the reproductive function.

Throughout the bryozoa the sexual elements are produced, as a rule, in the zoœcia and in connection with the polypides. Thus, Nitsche ('69) found that in *Bugula* the ova arise from the inner surface of the endocyst of the younger zoœcia. In the older zoœcia he found the spermatozoa and in still older ones, the fertilized ova. Vigelius ('84) reports that in *Flustra membranacea-truncata* the genital products, both male and female, also arise from the endocyst of the zoœcium, and Prouho ('92) in a series of observations upon the Ctenostomes, found essentially the same condition as far as the time and place of origin of the sexual elements are concerned.

More recently, Calvet ('00) has reported a series of observations upon no less than forty-four species of marine bryozoa. These studies have reference mainly to the Cheilostomata and the Ctenostomata, his study of the Cyclostomata having been very restricted. In one species of the Cyclostomata, viz., *Crisia denticulata*, he made some observations on the reproductive processes, corroborating the researches of Harmer on the fission of the embryo. In the list of species whose spermatogenesis he studied, he mentions two Cyclostomes: *C. denticulata* and *Tubulipora flabellaris*. In his discussion he makes no particular mention of them, however, merely including them in the list with others, in which he says the primitive sperm cells originate as in *Bugula sabatieri*, *i.e.*, in the vicinity of the funicular cord in the lower portion of the zoœcium. One can only infer that he made no investigation of the growing tips of these two species, and the study of the adult animals alone would certainly mislead one as to the time and place of origin of the spermatoblasts.

Calvet's study of ovogenesis in *Bugula sabatieri* reveals an

interesting similarity between the origin of the ova in that species and in *Crisia*. Thus, in the young tips, he finds large cells which he considers to be "éléments ovulaires." Furthermore, he finds these cells in a cavity of a branch, distal to the region where the polypide buds are found. He says: "Dans les blastozoïdes jeunes, soit par l'observation directe sur le vivant, soit par l'examen comparatif des coupes histologiques, on peut suivre pas à pas la genèse des différentes parties constitutives de l'ovaire adulte. Il n'est pas rare de rencontrer, parmi les éléments libres de la cavité d'un blastozoïde terminal renfermant un polypide à l'état de rudiment massif, un certain nombre de cellules qui, par leurs grandes dimensions et leurs caractères histologiques, se désignent déjà comme éléments ovulaires (Pl. V, fig. 7et9, *ovu*)." His description of these cells leaves no doubt that they are eggs, and his figures show the close resemblance between them and the ova found at the growing points of a colony of *Crisia* (Pl. XII, Figs. 13, 14, and 15). This writer regards the ova which he finds at the growing points of *Bugula* as exceptional, and not as showing the ordinary method of their development. When so found they constitute merely the "anlage" of the future ovary, and in no case does he find the mature ovary outside of a zoœcium containing a polypide. In this respect then, *Bugula* differs materially from *Crisia*, since in the latter genus the ova which appear among the free elements of the tips of the branches, constitute the ovaries, and it is here that the ovum matures, is fertilized, and unites with a young bud to form an ovicell.

There is much in confirmation of these observations on the early development of the genital products, and of their independence in their earliest stages, to be obtained from Harmer's investigations. That writer reports the finding of egg-like cells in the growing tips of *Crisia*, and says, "The fact that these eggs are commonly found in the growing points, leads me to suppose that several are produced in each fertile internode; apparently by a modification of the funicular tissue, and that their further development depends upon their entering into definite relation with a polypide bud." In *Tubulipora* ('98), he finds that eggs are abundant in the young lobes. He found them in many of the zoœcia in connection with polypides and polypide

buds of every stage of growth. In *Lichenopora* ('97), he found but one egg, as a rule, in each colony, and always in the second or third zoœcium, and when the polypide was very young. In all these cases he regards the egg as "probably differentiated *in situ* from the outer layer of a young polypide bud," or, "The eggs appear as part of the polypide bud." Or again, "The eggs (of *Tubulipora*) are developed at a very early stage by the polypide buds, as in *Lichenopora* and *Crisia*." Furthermore, he found an egg-like cell 9.6 μ in diameter at the growing margin of a colony of *Lichenopora*. He did not feel sure that this was normal, although as he says, it recalls the condition in *Crisia*. In his study of embryonic fission this observer made no special study of the origin of the sexual elements. He explains the occurrence of ova at the growing margin as due either to the productiveness of the young buds, or as an unusual, perhaps abnormal phenomenon.

In *Crisia* one fails even in the height of the breeding season to find even a rudimentary ovary within the individual zoœcia, or elsewhere. What becomes of the relatively large number of ova? Do they all reach maturity? If not, what is their fate? In answer to these questions it is to be said that all ova do not produce embryos. According to their fate they fall into three classes. The first (*a*), comprises the relatively small number that produce embryos within an ovicell. The second (*b*), includes the small number which reach a *partial development* within the zoœcia, and the third (*c*), includes the remainder which *fail* of development entirely.

It has already been shown in the case of the male colony that proximal to the region where the germ cells are formed is the budding region, and further that in order that the male germinal cells may complete their development, they must become united with a polypide bud. (Pl. XII, Fig. 4, *pd. bd. 1.*) In a similar manner, in order that an *ovum* may reach maturity, it is necessary that a union should be effected with a polypide bud. In his account of the reproductive processes in the Cyclostomata, Harmer ('93) has shown that a peculiar relation must exist between a bud and an ovum, in order that an ovicell should be formed. He says: "One of these (egg-cells) acquires a close

relation to the potential alimentary canal of the ovicell polypide," that is, to a bud which without the intervention of an ovum would have developed in the ordinary fashion. And further, "This potential alimentary canal grows round the ovum, losing its previous form and becoming a compact multinucleated follicle surrounding the egg . . . " The study of a series of sections from an ovicell-bearing colony, shows that the relations entered into by the bud and ovum are of two sorts, each producing opposite results. In the first the *ovum* develops, while the bud is aborted. This includes all the cases of the first class (*a*) as given above, and represents the only relation recognized by previous observers. In the other, the *polypide* grows to maturity while the ovum is aborted, and includes the second class (*b*) above. To distinguish between the earliest stages of these two possible relations is extremely difficult, if not impossible, since before the cells of the bud become somewhat differentiated, there is no criterion by which it can be certainly known whether or not an ovicell will result. Thus, in Pl. XIII, Fig. 14, an ovum (*ov.*) is shown in close proximity to a bud (*pd. bd. 1*), but the outcome of this relation cannot be predicted. Again in Fig. 15 several ova are seen in close connection with a group of small cells (*pd. bd. 2*), but whether or not there is here an incipient ovicell, cannot be asserted. Can the union indicated by the proximal polypide bud of this figure (Fig. 15, *pd. bd. 1*) be interpreted as the beginning of an ovicell? This bud consists of a long column of cells having a somewhat definite arrangement, and caught at its proximal extremity is a large ovum. This, for a time, was thought to represent an incipient ovicell, but the conditions shown in Fig. 16 reveal its true meaning. But one bud (*pd. bd.*) is represented in this figure, and this has reached a stage of development similar to that shown for the proximal bud of the preceding figure (Fig. 15, *pd. bd. 1*). If we compare the arrangement of the cells of the bud in these two cases, with buds which represent early stages of undoubted polypide formation, the resemblance is strong, and there can be no doubt that these are stages in polypide development. Thus in Pl. XII, Fig. 11, are shown several instances of the earlier stages in the development of a polypide. The cells in the upper portion of the bud

arrange themselves in parallel lines forming the incipient tentacles (*pd. bd. 1* and *3 in. tent.*), while those in the lower portion form into a hollow sphere to produce the cavity of the stomach (*st.*). The proximal bud of Pl. XIII, fig. 15 (*pd. bd. 1*), and the anterior bud of Fig. 16 (*pd. bd.*), represent a stage in the development of polypides identical with those in Fig. 11. The significance of the union of ovum and polypide in these two cases is further revealed by the polypide just proximal to the young bud (Pl. XIII, Fig. 16, *pd. 2*). Here attached to the cæcal end of the stomach of an adult polypide, is a veritable embryo (*emb.*) consisting of at least three cells. That these are blastomeres of an embryo, and not merely a bunch of ova, is shown by the condition of the nuclei. The two upper cells have apparently just completed their mitosis, and the nuclei are relatively small. The nucleus of the lower cell has lost its nuclear wall, and the cell is preparing for division. This case affords an explanation of those instances where an ovum is held by a delicate membrane at the proximal end of a column of cells, and represents a kind of union that may occur between a bud and an egg, but one in which *no ovicell results*. The next older polypide (*pd. 1*) possesses neither ovum nor embryo. Young embryos of two or three cells are not uncommon upon buds or young polypides near the growing points, although single ova attached to young buds and to adult polypides are of more frequent occurrence. This figure (Fig. 16) represents a typical section through the growing tip of a young colony. In the growing tissue, right and left, ova are more or less numerous. Proximal to this, the youngest bud frequently possesses an ovum, and below this, one or two polypides may carry a single ovum each, or a young embryo. The coexistence of polypide and embryo or ovum has not been previously noted in this subclass of bryozoa, and while it is probably an abnormal condition for *Crisia*, it is, perhaps, indicative of a more primitive method of reproduction. I have never observed this except at the height of the breeding season, when ova are being rapidly produced. In the older portions of the colony neither eggs nor embryos have been found, nor have larvæ been obtained, in any of the older zoœcia. These embryos, apparently, never attain complete development, but are absorbed.

This kind of union was not recognized by Harmer, and as a consequence the instances which he offers as probable early stages of an ovicell are somewhat doubtful ('93, Pl. 22, Figs. 1 and 2). This is especially true of Fig. 1, which is probably an instance of this second relation.

The partial development of an embryo in connection with a polypide is interesting for two reasons. In the first place, it probably points to a more primitive method of reproduction, and in the second place, it is important for the light it throws on the time and place of fertilization.

In regard to the indications of more primitive conditions, it is clear, aside from the question of the origin of the ova, that in *Tubulipora* (Harmer, '98) ova occur in many of the zoœcia. Moreover, in this genus any zoœcium may become an ovicell, and usually several zoœcia of a colony become thus transformed. In the constant occurrence of eggs in the individual zoœcia, and in the direct transformation of the latter into ovicells, *Tubulipora* shows the least specialized condition of any Cyclostome whose history is known. In *Lichenopora* an ovum is found only in that young zoœcium which becomes the ovicell of the colony, and which Harmer designates as the fertile zoœcium. In this case specialization may be considered to have gone a step further in setting off a certain zoœcium to perform the function of an ovicell, and perhaps to produce the single egg which comes to maturity. In *Crisia* specialization has proceeded so far that the ovicell is at no time a zoœcium, although from its position in the internode it must be considered homologous with one. While the ova in this genus are a colonial production and always originate at the anterior edge of the branch, they are occasionally found in the individual zoœcia. Such instances may be regarded as representing an early tubuliporidan stage, or possibly a more primitive stage in which each zoœcium brought at least one ovum to maturity.

In regard to the time and place of fertilization, it may be said that since *Crisia* is dicœcious the question arises as to the time when, and the manner in which the spermatozoa reach the ova. According to Harmer, fertilization probably takes place after the egg has been inclosed by its follicle and after the ovicell

has been started. He considers that the very thin wall of the anterior end of the ovicell is not impenetrable to the spermatozoa. If, indeed, the spermatozoa reach the ova at all, they must penetrate the tissues of the colony at some point. Whitman ('90) has shown that a method of impregnation somewhat similar to this is not uncommon in several groups of animals. In most of the cases he mentions the spermatozoa are forcibly injected through the cuticle, and wandering through the tissues, some succeed in reaching the ova. *Crisia* is covered with a calcareous layer which is pierced at intervals by pores that extend through the chitinous ectocyst beneath it. The epithelial cells of the body wall pass through these pores and spread out over the surface, forming a very thin layer upon it. These pores afford innumerable points where spermatozoa could effect an entrance. Moreover, near the growing tip the outer covering becomes thinner and the deposition of calcareous material does not keep pace with the growth of the branches, so that the growing points are covered with an extremely delicate chitinous layer only. Since, as has been shown, the ovaries are situated at the growing tips, it is practicable for fertilization to take place before, or at the time that the ovum becomes associated with the bud. The occasional occurrence of embryos in a zoœcium, as for example in the case shown in Pl. XIII, Fig. 16 (*emb.*), where cleavage has occurred, indicates fertilization thus early, as does also the early cleavage in an undoubted ovicell shown in Pl. XIII, Figs. 19 and 20. One of the blastomeres resulting from the first cleavage is shown in each of these figures (*bl.*). They are not surrounded by the cells of the polypide bud (*pd. bd.*), and yet the first division has taken place, so that cleavage occurs, apparently, at or before the time that the ovum is surrounded by the cells of the bud, and before the ovicell is formed.

The view that Harmer advances in regard to the time of fertilization is based upon his belief that the ovum is the product of the polypide bud ('97). He considers that only certain buds in each internode produce eggs, that these are equivalent to fertile polypides, and that they give rise to ovicells. The evidence from my own observations, however, proves that eggs are produced in every terminal internode, independently of either buds or poly-

pides, and that they become only secondarily united with buds. Moreover, it seems probable that *any* bud may form a union with an ovum, but that all such unions are not fertile, *i.e.*, do not produce embryos that give rise to larvæ. The view that fertilization may take place at a time earlier than that at which the ovicell is formed, and before the egg is surrounded by its follicle, is supported by the facts given above.

This brings us to the consideration of another possibility which correlates the probable degeneration of the male cells with a possible parthenogenetic development of the ovum. A most careful and thorough search has been made through both young and old portions of ovicell-bearing colonies for spermatozoa. None whatever were found, although their size is not so minute that they should be imperceptible with the high power of magnification used. The possibility of parthenogenesis has already been suggested by Smitt ('63), who, according to Claparède ('70) had observed the asexual development of the egg in the ovicells of *Crisia eburnea* and *C. aculeata*. Smitt's reason for supposing that the ova of several species of bryozoa develop parthenogenetically is mainly the failure to find spermatozoa. On this point Claparède remarks that from Smitt's account it seems probable either that the forms he reported upon are diœcious, or that parthenogenesis may occur in the bryozoa under certain circumstances. Of course, mere failure to find spermatozoa is insufficient ground upon which to base a belief in parthenogenetic development, and as a matter of fact, one of the species Smitt mentions, viz., *C. eburnea*, is diœcious. At the same time the evidence here given of degeneration of the testis adds weight to this suggestion, and the small number of spermatozoa compared with the vigorous growth of testis is not only remarkable, but may be correlated with the small number of ova that reach maturity either partial or complete. It is possible that this degeneration may be carried so far as to produce no mature spermatozoa whatever, or so few that their rôle in the economy of reproduction is reduced to the lowest degree.

Instances of the third class of ova (*c*), *i.e.*, those that fail of development, may be found in sections of the extremity of a branch where ova are frequently found in various positions,

sometimes upon the tentacle sheath of a developing polypide, sometimes lower down upon a septum, and sometimes free in the mesenchyme which fills the interior of the tip. In this last situation they frequently possess long processes which suggest that they have an amœboid motion. Their position, however, is to be attributed not so much to their own movement as to the fact that the tip has grown away from them, and has left them suspended in the network of interior cells. Pl. XIII, Fig. 17, represents a section in which two such ova have been thus left behind (*ov.*) and which, like those embryos which reach only a partial development, are absorbed. Measurement shows that the ova decrease in size as their distance from the growing point increases, and in the lower zoœcia no eggs are found, they having gradually disappeared.

A number of measurements of ova in various positions, *e. g.*, those in the ovaries, those on young buds or polypides, and those free in the different portions of the internode, shows that much variation in size occurs, but that these variations follow a regular law. Thus a gradual growth can be traced from the very small ova at the anterior edge of the tip, 5.4 μ in diameter, to older ones measuring 10.8 μ, 14.4 μ, and 18 μ. A parallel growth of the nucleus also occurs, those ova whose diameter is 10.8 μ possessing a nucleus of 7.2 μ, while those whose diameter is 14.4 μ and 18 μ have a nucleus measuring 10.8 μ.

The eggs attached to buds or polypides are, as a rule, larger upon the younger buds, and gradually diminish with the development of the bud. Instances are found where the ovum attached to the bud measures 21.6 μ with a nucleus 10.8 μ in diameter. A frequent size upon young buds is 18 μ, while upon older buds and polypides it diminishes to 11.7 μ and 10.8 μ, with nuclei varying in size from 9 μ to 7.2 μ. If the ovum develops even partially (Fig. 16, *emb.*), the blastomeres of the embryo, while large apparently, are smaller than the larger ova. In the instance shown in Fig. 16, *pd. 2*, the boundaries of the blastomeres are somewhat indistinct. One of them, however, measures 14.4 μ, while its nucleus is only 3.6 μ. Here, although the size of the blastomere as a whole equals that of some of the ova, the nucleus is much smaller. The outlines of

the others are too indisitnct for measurement. As a whole they are smaller than the upper blastomere, their nuclei measuring about 5.4 μ. The ova which fail of development and are free in the various portions of the internode, vary in size from 10.8 μ to 7.2 μ. Of these the smallest are invariably found at the greatest distance from the tip.

It is thus seen that ova increase in size from their origin at the anterior edge of the tip to the proximal border of the ovary. If, at this point, they unite with a bud, they may continue to increase somewhat in size. If the bud develops into a polypide, the ovum either becomes an aborted embryo or is absorbed without further development. Those ova which form no union with a bud are frequently found in the lower portion of an internode, much diminished in size. Those which develop in ovicells will be discussed later.

The data afforded by the preceding observations show that the time at which the genital products appear, both male and female, is much earlier than that at which the buds arise. The place of origin of each has also been shown to be different, and that the close relation existing between bud and ovum at a later period is secondary. Furthermore, it is shown that any bud may form a union with an ovum, *i.e.*, the possibility of a union between genital product and bud is the same for both males and females. As a matter of fact, however, every bud in a female colony does not unite with an egg, nor conversely does every egg succeed in uniting with a bud, a large number of ova undergoing degeneration. Of those ova which effect a union with a bud only a relatively small number give rise to larvæ, *i.e.*, become inclosed in ovicells. It seems probable, then, that certain buds only possess the possibility of developing into ovicells, viz., those which arise at that point in the internode where the ovicell is found. Any or every internode then has the possibility of being a fertile one. The questions are, Why does not every internode possess an ovicell? And why do some unions result in only a partial development of an embryo and no ovicell? What the determining factor is, is not known. A struggle seems to ensue between the two elements, bud and ovum, the one obtaining ascendency over the other. The result

may be due in part to the *time* at which the union is effected, *i.e.*, if the bud has already got started toward the formation of a polypide, the momentum of growth may be so great that the development of the egg has no power to change or hinder it. Whereas if the union takes place early enough, before bud differentiation has begun, the embryo gains the ascendency, and an ovicell results.

DEVELOPMENT OF THE PRIMARY EMBRYO.

The Ovicell.—Development of the embryo in *Crisia* takes place within a special structure, the ovicell. Smitt ('65) first called attention to the fact that the ovicell of *Crisia* develops according to the same laws as zoœcia, and Harmer has shown that in several genera of the Cyclostomata it is homologous with a zoœcium. The reasons for these conclusions are first, in *Crisia* the ovicell occupies a position in the internode similar to that of a zoœcium. In *C. eburnea* there are ordinarily seven zoœcia in an internode, so that ovicell-bearing internodes consist of six zoœcia and an ovicell, the latter taking the place of the second or third zoœcium.* Second, within the ovicell is found a bud which is equivalent to that found in a zoœcium. In the latter this develops into a tentacle sheath and the alimentary canal of a polypide, in the ovicell, into a tentacle sheath and the follicle inclosing the embryo. Third, in *Lichenopora* and *Tubulipora* the ovicell originates in an actual zoœcium. In the former it is the second or third zoœcium of the colony and functions as a brood pouch only after the degeneration of the first polypide; in the latter, any zoœcium may become an ovicell, and after it has already had one or two occupants.

Pl. XIII, Fig. 18, represents in optical section a decalcified tip containing a young ovicell (*ovl.*) in the so-called "funnel stage", in which is a very young embryo (*emb.*), and the beginning of the tentacle sheath (*tent.*). Starting with the chitinous articulation (*art.*) at the base of the internode, the ovicell is found in this instance to occupy the place of the third zoœcium.

*This statement may seem inconsistent with that made on p. 134 relative to the difficulty in locating early ovicell stages, but determinateness in the position of the ovicell is not accompanied by constancy in its occurrence, relatively few internodes possessing ovicells.

In the rear of the ovicell the continuation of the internode appears in the form of young buds. These would have eventually grown beyond the ovicell and have constituted the remaining zoœcia of that internode. Just what stage of development this embryo has attained, it is difficult to say, but judging from others of similar size and appearance it probably consists of three or four blastomeres.

Early Cleavage Stages.—It was remarked above that the earliest stages of ovicells are difficult to distinguish. In the sectioned material, no instance has occurred in which a single ovum is contained within an undoubted ovicell. There are many cases of juxtaposition of ovum and group of cells, but as has been shown the interpretation of this relation is not always possible. It is true that at an early stage an ovicell can be detected by its size, but on sectioning material that could be thus distinguished, cell division has always been found to have occurred. Since in *Crisia eburnea* the ovicell occurs in the proximal portion of the internode, usually in the place of the second or third zoœcium, it would seem relatively easy to secure the early stages by preparing in large numbers the young tips of colonies in active reproduction. This method was adopted, but without success in obtaining an undoubted ovicell containing an ovum previous to cleavage. In all of the earliest stages secured, the first cleavage at least had occurred, and there are reasons for supposing that cleavage usually occurs before the ovicell is definitely set off. Pl. XIII, Figs. 19 and 20, are consecutive sections of one of the three youngest ovicells obtained. The embryo consists of two blastomeres, one being represented in each figure (*bl.*). These latter are large ovum-like bodies imbedded in cells and lying distal to a mass of elongated cells which represent the polypide bud of an ordinary zoœcium (*pd. bd.*), and which seem to be arranging themselves around the embryo to inclose it. The cells of the embryo possess a large vesicular nucleus, and in size and appearance bear so close a resemblance to ova, that the question arises whether they may not be such. The strongest evidence that they are the result of cleavage is found in the relative size of nucleus and cell. Measurements of a large number of ova show that the ratio of the size of the

whole cell to that of the nucleus is 2:1.5 or less, whereas in the blastomeres it is 2:1 or more. This latter rule holds in the present case. Thus in Fig. 19, although the blastomere is as large as many ova, *i.e.*, 14.4 μ in diameter, its nucleus is only 7.2 μ, while in Fig. 20 the blastomere measures 9 μ with a nucleus of 3.6 μ. In the first the ratio is just 2:1, in the second it is slightly greater. Additional evidence that these bodies are not ova is afforded by the difference in their rate of growth since cleavage. In a second instance an ovicell in the same stage contained an embryo of two blastomeres still adhering to each other as if division had but recently occurred. The cells of this embryo are relatively very small, the two measuring 14.2 μ, about as much as a single ovum. The cells of the bud have much the same appearance and bear the same relation to the embryo as those shown in the bud of Figs. 19 and 20. That the latter represent an early stage in the development of the embryo is further shown by the fact that the blastomeres are not yet surrounded by the cells of the bud (*pd. bd.*). Nevertheless that some time has elapsed since cleavage occurred is shown again by the presence of the small cells between the blastomeres. Furthermore, the separation of the blastomeres shows that cell division takes place some time previous to or following very close upon the formation of the ovicell. In this ovicell there is yet no appearance of the tentacle sheath, the two lines of cells extending downward from the anterior border being those that form the vestibule (*vest.*).

A somewhat later stage of embryonic development is represented in Pl. XIV, Fig. 21. Here the embryo (*emb.*) contains at least three blastomeres which are not only surrounded by the follicle but are pushed apart and separated by the interior cells. The beginning of the tentacle sheath is shown in the layer of cells separating from the distal surface of the bud, the cavity formed between the outer surface of the bud and this layer (*tent.*) being the cavity of the tentacle sheath (*tent. cav.*). Here again the blastomeres have the same ovum-like appearance as in the two-cell stage, but they are smaller, the larger of them being 10.8 μ in diameter, and the other two about 7.2 μ. In this stage the cells between the blastomeres are smaller than those in

a similar position in the two-cell stage. The separation of the blastomeres and the interpolation of small cells is a characteristic of the early stages of *Crisia*, and in most older stages than the two-cell stage the blastomeres divide quite independently of one another. Pl. XIV, Fig. 22, represents a four-cell stage in which again are shown the separation of the blastomeres and the interpolation of the follicle cells (*sm. fl. cls.*). This ovicell is further interesting as showing the characteristics of the follicle cells. These now surround the embryo so that it lies in the center of a sphere consisting of a number of concentric layers composed of cells which form a net-work by the union of their protoplasmic processes (*fl. cls.*). In the interior of the spherical follicle the four blastomeres of the embryo may be distinguished by their larger size (*bl.*). The other cells of the interior (*sm. fl. cls.*) are of various sizes, those nearest the embryo being the smaller, those nearest the inner layer of the follicle, the larger. An examination of a large number of specimens shows that the multiplication of the small cells is accompanied by a diminution in number of the cells of the concentric layers. The former seem without doubt to be derived from the latter and to represent a stage in their absorption. Pl. XIV, Fig. 23, represents an embryo in the eight-cell stage, only four blastomeres being visible in this section. The separation of the cells of the embryo is clearly brought out, the blastomeres being perfectly distinguishable by their larger size and their different staining capacity. The increase in the number of small interior cells is noticeable as is also the decrease in the follicle inclosing the embryo.

This separation of the blastomeres continues to be a striking feature of the embryonic development of *Crisia* until about the twenty or twenty-four cell stage when the blastomeres unite to form a more or less compact ball. Harmer ('93, '97 and '98) has shown that it is characteristic of this and also of other genera of the Cyclostomata viz., *Lichenopora* and *Tubulipora*. In a recent paper, Braem reports a somewhat similar method of cleavage for *Plumatella*. According to this writer the egg of *Plumatella* consists of two quite distinct parts, an outer granular zone, and an inner zone containing the nucleus.

It is the latter only which takes part in cleavage and from which the blastomeres are formed. At the first cleavage the plane of division does not pass entirely through the egg, even of that part out of which the embryo is formed, and as a consequence the first two blastomeres, while being connected at one pole, fall asunder at the other. The undivided portion, called the middle piece (mittelstück), remains intact through the two, four, and eight-cell stages, while the blastomeres are widely separated at the animal pole. In the meantime the granular zone disintegrates more or less, its granules become larger, and nuclei appear between the free ends of the blastomeres. It is in the sixteen-cell stage that the resemblance between the embryos of *Plumatella* and *Crisia* is closest. At this time the middle piece disappears and the blastomeres being set free completely separate from each other. They continue to increase in number, although not regularly, while in the spaces between them are numbers of small cells. With further increase in the number of blastomeres, the small cells gradually decrease in number until, in the twenty-four cell stage the blastomeres having united into a ball, the small interpolated cells disappear almost entirely. From this point development proceeds in the regular manner. A comparison of the series of figures I to V in Fig. 104, Pl. IV, of Braem's paper, with Figs. 22, 23, and 24 of this paper will show the similarity of the cleavage in the two cases. The resemblance consists not only in the separation of the blastomeres but in the appearance between them, as if shoving them apart, of numerous small cells resembling those similarly situated in the embryo of *Crisia*. The function of these cells in both cases is probably identical, *i.e.*, they serve as nourishment for the embryo. As in *Crisia* the interpolated cells gradually disappear and the blastomeres unite at about the twenty or twenty-four cell stage into a solid ball.

The Ball Stage.—From the twenty-cell stage onward the embryo of *Crisia* forms, as has been said, a more or less compact ball. Pl. XIV, Fig. 24, represents an embryo measuring 43 μ in diameter and containing from sixty to seventy blastomeres which have united into a ball, although still surrounded

by the original follicle (*f.*). In this case the small follicle cells have not disappeared but may be seen packed together in the space around the embryo in the cavity of the follicle (*sm. fl. cls.*). Numbers of mesenchymatous cells forming a net work are present in the cavity of the tentacle sheath.

Pl. XIV, Fig. 25, represents a much older stage. This embryo is a compact ball with a well differentiated outer layer. Its greatest length is 150 μ, while the size of the separate cells varies from 5.4 μ to about 8 μ or 9 μ. At higher magnification these larger cells are shown to be in division, but mitosis does not seem to occur more actively in one part of the embryo than another. The absence of the follicle is very noticeable at this stage, but that its loss is probably more gradual than has so far been indicated, is shown by Pl. XIV, Fig. 26. This is a section of an ovicell of *Crisia occidentalis*, in which the embryo has attained about the development of that in Fig. 25. Here a portion of the original follicle remains in the chain of cells lying below the embryo (*fl. cls.*). These cells occupy the position and have the appearance of the follicle cells of other embryos, possessing the enlarged nuclei with scattered chromatin granules. In this ovicell a number of other cells are present below the embryo which represent a possible source of a second follicle (*sec. fl. cls.*). These latter are most numerous in connection with a chitinous tube (*chi. t.*) which extends from a septum (*sep.*) below the embryo to the base of the ovicell. In development this tube begins as a layer of chitin below the young embryo then consisting of only a few cells. Later the chitinous layer becomes more extensive and assumes a cone shape, the apex of which, with the continued growth of the ovicell, extends to the proximal extremity of the ovicell. Meantime a chitinous ring forms immediately below the embryo (*chi. r.*), dividing the ovicell into two parts. The tissue lining the ovicell is continued over the septum into the tube, and throughout its extent and in close connection with it there appears numerous large cells often possessing two or three nuclei, resembling the giant cells (*gi. cls.*) found in the ovicell of *C. ramosa*. The interior of the tube is filled with a net-work of deeply staining cells that extends above the septum and around the embryo. The chitinous ring or

septum,* (*chi. r.*) probably serves as a supporting structure to keep the embryo from passing downward into the narrow portion of the ovicell, but the whole tube seems to be related to the great development of the second follicle in this species. In *C. eburnea* the follicle of the adult ovicell consists of a relatively small number of cells scattered among its contents (Pl. XV, Fig. 28). In *C. occidentalis*, however, the second follicle is a mass of cells in which the embryos and larvæ are imbedded (Pl. XV, Fig. 29). With the disappearance of the spherical follicle and the appearance of a second follicle, the embryo attains a relatively enormous size before budding begins.

The Secondary Embryos.—An early budding stage is shown in Pl. XIV, Fig. 27. This is drawn to the same scale as Figs. 25 and 26, and a comparison with these two figures will give an idea of the great size which the embryo attains, this one being 200 μ. in its longest diameter. As the embryo increases in size it comes to occupy a higher position in the ovicell, moving upward apparently to the point where the walls are more widely expanded. This is especially noticeable in *Crisia cornuta* where the ovicell is widest at the distal end. The embryo is not anchored in any way in *C. eburnea*, and is often found at the top of the ovicell close against the valvular closure (Pl. XV, Fig. 28, *prim. ebm.*) Buds are formed at various places on the body of the embryo. In the case represented in Fig. 27, two somewhat irregular processes project distally, from the extremities of which small portions are constricted (*sec. emb.*). These are not the only budding regions, however, for on other parts of the surface outgrowths occur which as other sections reveal, are incipient buds (*in. bd.*) At the proximal extremity there are a few cells which the examination of preceding sections shows belong to another bud (*sec. emb.*). There are instances also where the first buds are constricted from the extremities of long arms extending proximally through the whole length of the ovicell. The primary embryo frequently possesses a somewhat rounded triangular form, and the buds are

*The septum found in the base of the ovicell of *C. occidentalis* is probably homologous with the chitinous articulation occurring on each zoœcium of this and the related species, *C. geniculata* and *C. cornuta*. Evidence for this homology will be given in a later paper.

given off at the apices. This is contrary to the observations of Harmer who finds that the primary embryo of *Crisia ramosa* buds only at the distal extremity. Calvet ('00) also represents the same condition for *Crisia denticulata*.

The buds of the primary embryo, from whatever portions of the body they arise, constitute the secondary embryos and from them the free swimming larvæ develop. When first set free the secondary embryos of *Crisia eburnea* consist of a small number of cells united into a solid ball, and varying in size from 25 μ to 35 μ in diameter, containing approximately from 55 to 65 cells. Redivision of the secondary embryos has not been observed in this species. In *Crisia occidentalis*, however, there occurs not only the formation of secondary embryos by budding, characteristic of *C. eburnea*, but also, in some cases, a redivision of these to form tertiary embryos. In these cases the primary embryo breaks up into large masses of cells, the secondary embryos, which in turn, become budding centres, from which tertiary embryos arise, these ultimately becoming the ciliated larvæ. This is illustrated in Pl. XV, Fig. 29, which represents a section of an almost adult ovicell of *C. occidentalis*. On examination of the series of sections to which this figure belongs, it is seen that the ovicell contains a few fully developed larvæ (*lar.*). The presence of these indicates that the primary embryo had budded off a few secondary embryos at an early period, and that, later, it divided almost simultaneously into a number of embryos. Some of these may have undergone no further division, while others notably the masses *a* and *b*, divided into tertiary embryos. The method of division in these cases is different from that which takes place in *C. eburnea*, although the result is the same. At the point where the division is about to occur, the nuclei arrange themselves into two linear series parallel to each other, or almost so. In this way two or more masses are formed which round up, separate from each other, and become the tertiary embryos. Many instances of this method of division are shown in the series of which Fig. 29 is a section. In the mass of cells, *x*, such a process is taking place. Pl. XV, Fig. 30 represents an embryonic mass, taken from another ovicell, showing two tertiary embryos (*ter. emb.*) which are forming from a large secondary

embryo. In *C. eburnea*, neither in the primary embryo nor in the buds when first set free, is there any differentiation into cell layers. As the primary embryo increases in size, the cells upon the surface become more compactly arranged, the inner cells forming a loose, spongy mass. The secondary embryos of *Crisia denticulata*, according to Calvet, possess two distinct layers, an outer containing large nuclei, and an inner containing much smaller nuclei surrounding a central cavity. This is true even before the buds are detached from the parent. This central cavity persists and forms part, at least, of the general cavity of the first individual of the new colony. When the secondary embryos of *Crisia eburnea* are first set free they do not differ histologically from the primary embryo. No cavity is present, the cells being heaped together in a somewhat irregular way. When a cavity appears it is not at first lined by a distinct layer of cells as is the case in *C. denticulata*. By the time the ovicell has completed its growth it is filled with larvæ of various sizes and in various stages of advancement. Fig. 28 is a section through an ovicell which is almost mature, *i.e.*, one in which the larvæ outnumber the embryos and will soon be set free. In this instance many of the larvæ have attained their full development and are confined in their narrow quarters only until the valvular membrane can be perforated. The larger larvæ possess long cilia, which fact suggests that either they move bodily through the ovicell, or that the vibrations of their cilia set up currents which carry the smaller bodies about. It is not uncommon to find the secondary embryos remote the length of the ovicell from the primary embryo, showing that the contents of the ovicell must have been in motion during life. The size of the larvæ seems to be pretty constant, at least in a given species. Those of *C. eburnea* measure about 86 μ in diameter, while those of *C. occidentalis* are somewhat larger, measuring 107 μ. The opacity of the living ovicell prevents any study of the living contents while the ovicell is intact. But if a living ovicell be crushed in a drop of sea water, a very interesting scene is presented. The larvæ dart away and swim about with great activity. Smaller ciliated balls move about in clusters. The color of the whole mass, larvæ, embryos, and cellular

tissue, is yellow. Perhaps the most interesting sight is the primary embryo which floats out with the rest of the material and frequently beeomes isolated. It may easily be obtained by the dissection of a living ovicell, or from a stained decalcified ovicell dissected in a drop of oil. In the latter case the embryo is a more compact and clearly defined mass than in the former, but the characteristic features of both are the same. Projecting from the surface in various directions protuberances appear which are the buds of the secondary embryos.

Near the top of the ovicell represented in Fig. 28, the primary embryo appears much reduced in size, but still budding actively. As budding continues the primary embryo decreases in size, both as a whole, and in the size of its individual cells. This may be seen by comparing Figs. 27 and 31, the latter representing the primary embryo of Fig. 28 drawn to the same scale as that in Fig. 27. This, as has been said, measures 200 μ in length, while the older embryo (Fig. 31), measures but 71 μ in length. In the older embryo cell boundaries are less distinct, and the cells are more closely massed together. In examining a number of ovicells, primary embryos are frequently found much smaller than this, and much smaller than the contained larvæ. Thus in one instance the primary embryo measures 50 μ and the adult larvæ 86 μ. This ovicell contained a number of secondary embryos 25 μ in diameter. The secondary embryos in the older ovicells average slightly smaller than those in the younger. It seems extremely probable for several reasons that the primary embryo is completely used up in the process of budding. Evidence for this is found in the gradual decrease in size of the embryo resulting from its continued activity in budding. Again, the instance of *Crisia occidentalis* (Fig. 29) in which the primary embryo divides into a large number of secondary and tertiary embryos, so that no one of the masses present can be called the primary embryo, and in which each mass of cells is apparently either redividing or is transforming into a larva, is strong evidence that no portion of the original embryo is left over. Further, complete series of sections of ovicells are obtained in which no primary embryo can be found, although larvæ and half grown, secondary embryos are abundant, and the aperture of the ovicell

is still unperforated. Finally, although empty ovicells are remarkably scarce, yet in one instance at least, a complete series was obtained which possessed neither larvæ, nor embryos, the interior containing nothing but a fine network and some degenerated cells. The evidence seems to be conclusive, then, that the whole of the primary embryo is converted into larvæ.

The number of larvæ to which a colony of *Crisia* gives rise is probably not less than is produced by other bryozoa although *Crisia* produces few mature eggs. As far as the evidence from my observations is concerned all the larvæ found in the ovicell, arise from one egg. Both Harmer and Calvet, however, believe they have evidence that more than one ovum may develop simultaneously within a single ovicell. Harmer ('97, Pl. 9, Fig. 25), represents two young embryos whose blastomeres are still separated, which he considers are the result of the development of two eggs. While this may be true, there is a possibility that the conditions presented may have resulted from the blastomeres of the two-cell stage of a single ovum having become so widely separated that each has gone on to develop into a separate embryo. The numerous recent experimental demonstrations of the power of independent development possessed by the blastomeres, and this too, in ova whose blastomeres normally retain their connection with one another, renders this hypothesis the more probable. Calvet figures a similar condition (Pl. 10, Fig. 15) which he considers affords undoubted evidence of the presence of two ova and of their simultaneous development within a single ovicell. Here again the facts may be differently interpreted. The two embryos may represent the individual development of two blastomeres which had become separated in the two-cell stage and had not reunited, or it may be an instance of a condition similar to what occurs in *Crisia occidentalis*. The two large masses, the two so-called primary embryos, may be two secondary embryos, and the smaller masses arising from these, may be tertiary embryos. The production of tertiary embryos is reported for *Lichenpora* and *Tubulipora*, but has not been previously found in *Crisia*. In the species in which it undoubtedly occurs, *Crisia occidentalis*, there is more or less variation, and it will not be surprising to find it in all species of the genus.

The protection and nourishment afforded the embryo of *Crisia* are typical of the Cyclostomata, and are paralleled to a certain extent among the Ctenostomata and the Phylactolæmata. According to Prouho, the Ctenostomes are, as a rule, viviparous, the different genera showing degrees of this condition varying from the primitive state exhibited by *Alcyonidium duplex*, where the young are sheltered during a portion of their development only, to that found in *Pherusa tubulosa*, for example, where several embryos develop in the tentacle sheath of a degenerated polypide. Joliet ('77), who studied the living animal, has given the most detailed account of the process. He shows that in *Valkeria cuscuta*, another Ctenostome, upon the degeneration of a polypide there appears in the zoœcium both an egg and a new bud. The latter grows into an immature polypide, but develops a tentacle sheath and the muscles belonging thereto. The small polypide soon degenerates while into the newly formed tentacle sheath the egg finds its way, and there develops into an embryo and ultimately into a larva. In both *Crisia* and *Valkeria* the development of the embryo is accompanied by the destruction of the polypide, and in both the embryo develops inside of the tentacle sheath newly produced to receive it, in the one case in a highly modified zoœcium, in the other, in an old unmodified one.

The developmental processes of the Phylactolæmata as exhibited by *Plumatella* show a closer resemblance in some respects to those of *Crisia*. According to Braem an ovary and a bud develop simultaneously on the body wall, the bud differing from an ordinary polypide bud in the possession of a high columnar layer and a flattened mesodermal layer. One of the cells of the ovary grows larger than the others, and partly by increase in its size, partly by pressure from behind, it approaches the side of the bud, pushes through it and becomes enveloped by it. This bud which according to Braem, Kraepelin ('93) and others is homologous with an ordinary polypide bud, now performs the function of a broodsac or oœcium, and shelters the embryo until it develops into a larva. The origin of the ovary of *Plumatella* appears to be similar to that in *Crisia* in its independence of a polypide. The suggestion of Braem, however, in regard to the relation sustained by the ovary of *Plumatella* and the bud which

forms the œcium is probably true, viz., that ovary and bud together constitute the equivalent of a sexual animal, the nutritive portion of which, the polypide, has undergone a change of function. The complete envelopment of the egg by a polypide bud is similar in the two cases, but in *Plumatella* this bud is set off structurally at an early stage, whereas in *Crisia* any bud may be thus set apart, no structural difference between it and an ordinary bud being at first discernible.

In its main features, the processes of embryonic fission as described by Harmer for *Crisia ramosa* and other Cyclostomes have been confirmed by this investigation, while certain additional facts and individual variations have been noted. Observations have also been made on the origin of the sexual elements and their secondary union with the polypide buds. The results may be summarized as follows:

1.—In the genus *Crisia* the sexual elements are produced in both male and female colonies, at the edge of the growing tips of the colony. The germ cells arise from the mesodermal layer, and are differentiated at a point anterior to the budding zone, and at a time earlier than the origin of the buds.

2.—In the male colonies of *Crisia eburnea* a few of the primitive germ cells attach themselves to each bud as it arises, and these form the beginning of the testis. In a majority of cases degeneration of the testis probably occurs before the spermatozoa become mature.

3.—In the female colonies the ovaries are produced at the anterior edge of the young tips. As in the male colonies, in order that the germ cells may reach maturity, it is necessary that they unite with a polypide bud. In this case one of two results may follow:

a.—The ovum may develop into an embryo, while the polypide bud as such, becomes aborted.

b.—The polypide bud may develop, while the ovum either degenerates at once or soon after it has passed through the early cleavage stages.

Many ova are produced which never form a union with a polypide bud. These soon degenerate.

4.—From the time the ovum leaves the germinal epithelium there is a steady increase in its size until it reaches the boundary of the budding region. If here it forms a union with a polypide bud, the size increases somewhat until division occurs. If after this union is effected, the polypide bud develops, the ovum gradually grows smaller. Those ova which fail of development, decrease in size as they become more remote from the ovary.

5.—Fertilization, if it occurs, takes place before or near the time at which the union of bud and ovum is effected. In view of the probable degeneration of the testis the possibility of parthenogenetic development is suggested.

6.—During its development the embryo within an ovicell becomes gradually inclosed by the bud which forms into a spherical follicle consisting of several concentric layers of cells.

7.—A characteristic feature of the early cleavage of *Crisia* is the complete separation of the blastomeres. This continues up to the twenty or twenty-four cell stage when the blastomeres unite into a more or less compact ball.

8.—The separation of the blastomeres is accompanied by the penetration between them of numbers of small cells, and by the diminution of the concentric layers of the follicle. With the continued growth of the embryo, the follicle being absorbed by the embryo gradually disappears.

9.—The primary embryo attains a size many times that of the original ovum before it divides to form the secondary embryos. In *C. occidentalis*, the secondary embryos divide to form tertiary embryos which develop into ciliated larvæ. At the close of its proliferation the primary embryo itself becomes a larva.

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LIST OF ABBREVIATIONS USED IN THE PLATES.

art.—articulation.
b. b.—brown body.
b.—proximal extremity of a zoœcium.
ba. cls.—ball of cells.
bl.—blastomere.
chi. r.—chitinous ring.
chi. t.—chitinous tube.
d.—dorsal side of internode.
de. cls.—degenerated cells.
de. pd.—degenerated polypide.
ec. cls.—ectodermal cells.
emb.—embryo.
fl.—follicle.
fl. cls.—follicle cells.
ger cls.—germinal cells.
gi. cls.—giant cells.
gr. tis.—growing tissue.
in. bd.—incipient bud.
in. tent.—incipient tentacles.
l.—left side of the internode.
lar.—larva.
m.—membrane.
mes. cls.—mesodermal cells.
mes. tis.—mesodermal tissue.
or.—ovum.
orl.—ovicell.
ovy.—ovary.
pd.—polypide.
pd. bd.—polypide bud.
prim. emb.—primary embryo.
r.—right side of internode.
re. pd.—regenerating polypide.
sec. emb.—secondary embryo.
sep.—septum.
sm. fl. cls.—small follicle cells.
st.—stomach.
t. cls.—cells of the tube.
tent.—tentacle.
tent. cav.—cavity of the tentacle sheath.
ter. emb.—tertiary embryo.
tes.—testis.
tet.—tetrads.
v.—ventral side of the internode.
vest.—vestibule.
z.—zoœcium.

All drawings made with the aid of a camera lucida, and all figures except 1 and 18, by the use of Zeiss oculars and objectives.

PLATE XII.

Fig. 1.—Portion of a young decalcified internode of *Crisia eburnea* showshowing the growing tissue (*gr. tis.*), the budding region (*pd bd.*), and the alternate arrangement of the zoœcia (*z.*).

Fig. 2.—Section from the tip of a male colony, close to the edge, right or left, showing the character of the cell layers, the small round ectodermal cells (*ec. cls.*), the larger mesodermal cells (*mes. cls.*), and a few cells of the germinal epithelium (*ger. cls.*). × 600

Fig. 3.—Section from the same series as the preceding, showing practically the same cell layers at a point nearer the middle of the tip where the ectodermal cells are thinning out and are becoming elongated (*ec. cls.*). 600

Fig. 4.—Section from a male colony through the budding region. In the angle toward the left edge of the branch, are a number of germinal cells (*ger. cls.*). Proximal to this is a young polypide bud (*pd. bd. 2*), still lower down is an immature polypide (*pd. bd. 1*) possessing a stomach (*st.*), and an incipient testis (*tes.*). 600

Fig. 5.—Two spermatozoa from a ripe testis of *C. eburnea*. 2500

Fig. 6.—Section of a zoœcium from a male colony showing a regenerating polypide (*re. pd.*), and below this a "brown body" (*b. b.*) extending to the base of the zoœcium. The brown body consists of a homogeneous mass of yellowish brown degenerated cells, the remains of the polypide (*de. pd.*) and the testis (*de. tes.*). × 600

Fig. 7.—Section through a zoœcium containing a normal testis. Distally, the stomach (*st.*) of the polypide is shown, while extending into the base of the zoœcium is the testis (*tes.*) in which the cells are arranged in scattered groups of various sizes. Numerous groups of four nuclei (*tet.*) are visible. × 600

Fig. 7A.—Group of four nuclei (*tet.*) in a mass of cytoplasm. × 2500

Fig. 8.—Section from the growing tip of a female colony showing the two cell layers of the body wall, the outer or ectodermal layer (*ec. cls.*), consisting of small round cells, the inner or mesodermal layer (*mes.. cls.*) consisting of larger cells, part of which gives rise to the germinal epithelium (*ger. cls.*), part to the spindle-shaped mesenchymatous tissus (*mes. tis.*). × 600

Figs. 9 and 10.—Serial sections from the same tip as the preceding. The ova are accumulated in the corners (*ger. cls.*). × 600

PLATE XII.—*(Continued.)*

Fig. 11.—Section through the bud forming region of a female colony, showing the relation of the polypide buds and the germ cells. The latter (*ger. cls.*) are differentiated at a point anterior to that where the buds form. Four buds are shown, in the older of which (*pd. bd. 4*) the cavity of the stomach has formed (*st.*). The cells above the stomach are arranged in somewhat regular rows, and represent incipient tentacles (*in. ten.*). These are again shown in the third polypide bud (*pd. bd. 3, in. ten.*). × 600

Fig. 12.—Section from the ventral side of a female colony, showing the cells of the zoœcial wall, the outer layer of a polypide bud (*pd. bd.*) lying close to the septum (*sep.*) which separates two zoœcia. The germ cells (*ger. cls.*) are prominent in the germinal tissue (*ger. tis.*). × 600

PLATE XIII.

Fig. 13.—A section which follows the preceding in consecutive order, representing a portion of the same septum (*sep.*) and of the same polypide bud (*pd. bd. 2*). There are shown besides a portion of another bud (*pd. bd. 1*) and numerous large ova constituting an ovary (*ovy.*). × 600

Fig. 14.—A section following the preceding in consecutive order, showing a portion of the septum (*sep.*), and in the cavity of the branch a large ovum (*ov.*) in close proximity to a polypide bud (*pd. bd.*). × 600

Fig. 15.—Section through the tip of a female colony, representing a part of an ovary (*ovy.*), a few cells of a young bud (*pd. bd. 2*) and a portion of an older bud (*pd. bd. 1*) to the proximal extremity of which a large ovum is attached (*ov.*). × 600

Fig. 16—Section through the tip of another colony, showing two ova in the germinal epithelium of the anterior edge (*ov.*), a polypide bud (*pd. bd.*) with an ovum attached to its proximal extremity. In the next older zoœcium is an adult polypide (*pd. 2*) with a small embryo (*emb.*) attached to the cæcal end of the stomach, and in the succeeding zoœcium is a still older adult polypide (*pd. 1*). × 600

Fig. 17.—Section through the tip of a female colony showing two ova (*ov.*), which are attached by long processes to the interior the branch. These have formed no union with a bud and would have degenerated. × 600

Fig. 18.—Decalcified internode of *Crisia eburnea*, containing an ovicell (*ovl.*) in an early stage of development. At the proximal extremity is the articulation (*art.*), by which the internode is connected with the branch. Arising from the articulation are two zoœcia (*pd. 1* and *pd. 2*), while the ovicell takes the place of the third zoœcium. At the distal extremity of the branch, two or three buds are forming (*pd. bd.*). The ovicell contains a young embryo (*emb.*), and a tentacle sheath (*tent.*).

Fig. 19.—Section of a young ovicell, containing an embryo in the two-cell stage. This figure contains but one blastomere (*bl.*), not yet surrounded by the cells of the polypide bud (*pd. bd.*). × 600

Fig. 20.—Section immediately following Fig. 19, showing the second blastomere (*bl.*) of the embryo, a portion of the elongated cells of the polypide bud (*pd. bd.*), and the beginning of the vestibule (*vest.*) × 600

PLATE XIV.

Fig. 21.—Section of an ovicell of *C. eburnea* containing an embryo in the three-cell stage. The outer layer of cells represents the tentacle sheath (*tent.*), and the cavity between it and the follicle (*fl.*) is the cavity of the tentacle sheath (*tent. cav.*). × 600

Fig. 22.—Another four-cell stage of *C. eburnea*, in which the blastomeres are separated (*bl.*) and between them are numerous small cells (*sm. fl. cls.*). The spherical follicle (*fl. cls.*) is diminished, the tentacle sheath is well developed (*tent.*), and below the embryo in the proximal portion of the ovicell are numbers of mesenchymatous cells (*mes. tis.*). × 600

Fig. 23.—Section of an ovicell showing four blastomeres of an embryo in the eight-cell stage. The concentric layers of follicle have decreased (*fl. cls.*), while the small cells (*sm. fl. cls.*) interpolated between the blastomeres have greatly increased. × 600

Fig. 24.—Section of an ovicell containing an embryo whose blastomeres have united to form a ball (*emb.*) which is still surrounded by the follicle (*fl. cls.*). Close to the embryo are a number of the small follicle cells (*sm. fl. cls.*). × 600

Fig. 25.—An advanced stage in ovicell and embryo formation. The follicle cells have disappeared, and within the tentacle sheath above and below the embryo are a number of cells of the mesenchyme (*mes. tis.*). × 600

Fig. 26.—Section of a ball stage of *Crisia occidentalis* representing an embryo at about the same stage of advancement as that in the preceding (Fig. 25). A portion of the original spherical follicle yet remains (*fl. cls.*). Below the embryo is the chitinous septum (*chi. r.*), separating the ovicell into two parts. The chitinous tube (*chi. t.*) contains large numbers of cells forming a network. Among them are numbers of multinucleated or giant cells (*gi. cls.*). × 600

Fig. 27.—Section of an ovicell of *C. eburnea*, containing a budding embryo (*prim. emb.*), and a number of secondary embryos (*sec. emb.*). At various points on the surface of the primary embryo are a number of projections, indicating the formation of buds (*in. bd.*) or secondary embryos. The follicle is represented by a number of scattered cells (*fl. cls.*). The tentacle sheath is intact (*tent.*). × 300

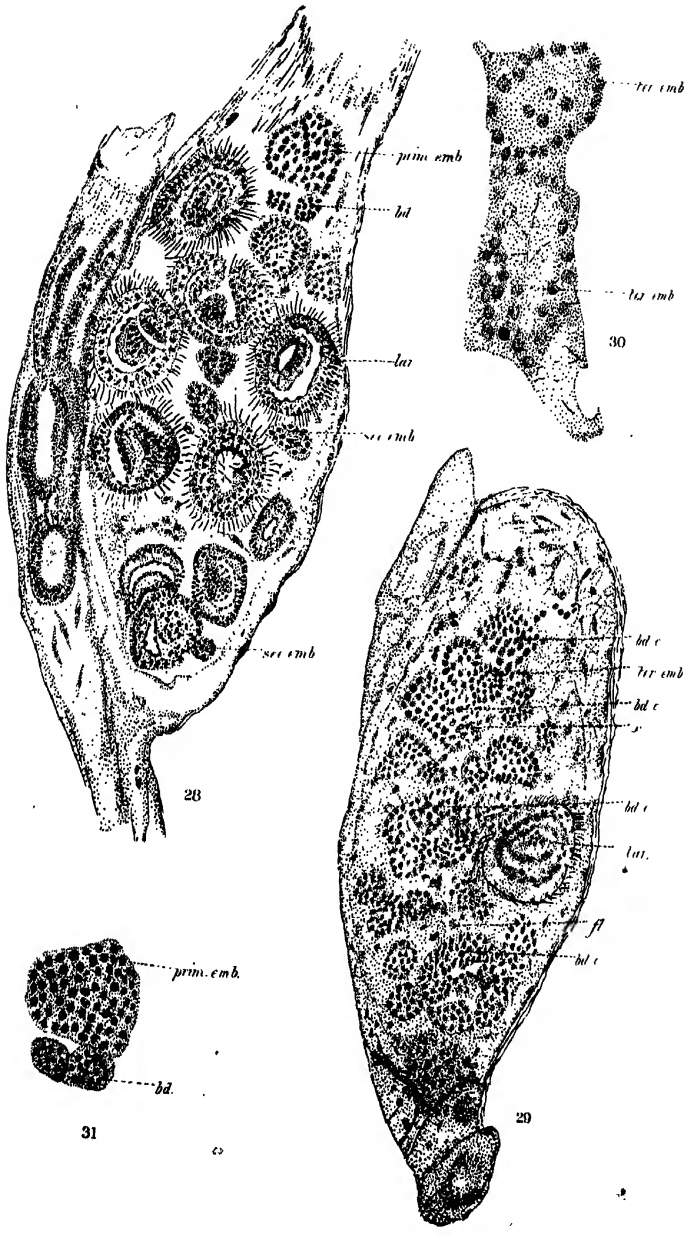
PLATE XV.

Fig. 28.—Section of a mature ovicell of *C. eburnea*, in which the larvæ outnumber the embryos. The primary embryo (*prim. emb.*) lies at the distal end of the ovicell, still giving off buds (*sec. emb.*). × 200

Fig. 29.—Section of an ovicell of *C. occidentalis*, showing the formation of tertiary embryos (*ter. emb.*), and the large amount of follicle (*fl.*). Tertiary embryos are forming from a number of budding centers (*bd. c.*), which are large secondary embryos. × 250

Fig. 30.—A single budding centre or secondary embryo from another ovicell of *C. occidentalis*, in which the two tertiary embryos (*ter. emb.*) are forming. × 2500

Fig. 31.—The primary embryo of Fig. 28 drawn to the same magnification as that of Fig. 27, to show the reduction in size of the former. × 300



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CORRELATED PROTECTIVE DEVICES IN SOME CALIFORNIA SALAMANDERS

BY

MARIAN E. HUBBARD

The observations recorded in these notes were carried on in the laboratory of Zoology at the University of California. I am especially indebted to Professor Ritter for his kind and suggestive direction of the work. I wish also to express my thanks to Professor Charles A. Kofoid, to Dr. Harry B. Torrey and to Mr. Calvin Esterly of the Department of Zoology of the University, for kindly assistance; also, to Mr. Leverett Mills Loomis, Director of the Museum of the California Academy of Sciences, to Dr. John Van Denburgh, Curator of Herpetology, and to Miss Forbes, Assistant Librarian of the same institution, for the use of specimens and the library of the Academy. I am furthermore indebted to Dr. Lorenza Yates, President of the Society of Natural History in Santa Barbara, for the use of specimens in the collection of that society.

Much has been written on the various skin glands of the Batrachia. Leydig, Engelmann, Wiedersheim, Zalesky, Schulz, Heidenhain, Eberth, Nicoglu, Capparelli and many others,* are among the investigators who have devoted more or less attention to the problems in this field, and Hoffmann, Nicoglu, Fatis, Wiedersheim and Boulenger in particular have given us valuable reviews of the literature and *résumés* of our knowledge.

*See bibliography at end of this paper for a somewhat more extended list of these investigators.

Beyond the questions of the structure and development of the glands, of their differentiation into such types as serous and mucous, of the chemical nature and physiological effects of the secretions, lies the fundamental problem of the *rôle* of the glands in the economy of the animals. This last problem has been much less studied than the others.

Some doubt is now being cast on the theories of protection in the animal kingdom that have been most in favor for half a century. Many structures and products of the organism hitherto regarded as sufficiently explained when they have been shown to be defensive are seen to owe their existence to more direct and simpler causes, as, for example, physiological activity, the effects of climate and other environmental influences, etc., and consequently if protective at all are often so only secondarily.

The study, the results of which are embodied in these notes, at first aimed merely at finding the nature of the enlarged condition of the tail in one species of salamander, *Plethodon oregonensis*. As the work advanced, however, its scope broadened until it touched upon the general problem of animal protection, the particular aspect of the problem chiefly involved being that of the correlation of protective devices.

The salamanders upon which observations have been made are *Plethodon oregonensis* Girard, *Diemyctylus torosus* Esch., and *Batrachoseps attenuatus* Esch. A fourth species, *Autodax lugubris* Hallow., occurs commonly at Berkeley, but does not enter into these notes because of the difficulty of obtaining specimens at the time the work was being done. Batrachoseps and Diemyctylus are very abundant, the former living under stones, old boards and rotting logs in damp, shady places, while the latter is found almost everywhere, but is especially given to congregating in the water of reservoirs and in quieter places in streams. Batrachoseps has a habit of burrowing in the soil, almost like an earthworm. Plethodon, though not so abundant as the other two species, is not uncommon. Like Batrachoseps it is found under rocks, logs and old boards, in moist, shady places. Neither of the last two species appears ever to enter the water.

Diemyctylus is diurnal in its habits, while Plethodon is nocturnal. I have never found a specimen of the latter abroad in

the daytime. Though conspicuous, it remains quiet when discovered under boards or rotting logs, not writhing or jumping about as Batrachoseps frequently does. Several times at night individuals in the terrarium were found alert and walking about, but the approach of the light soon sent them back to their hiding places beneath the bark and stones.

As stated before, this work began with the study of the tail of *Plethodon oregonensis*. This member in the great majority of cases is enlarged, the swelling being marked off from the rest of the body by a constriction just behind the anus. Out of sixteen individuals direct from the field fourteen were distinguished in this way, one of the exceptions being an immature individual, the other full grown. Of sixteen museum specimens fifteen showed the tail enlarged to a greater or less extent. The extreme of the swollen as compared with the unswollen condition is well shown in fig. 1, Pl. XVI. The tail segment in which the condition occurs is shorter than those immediately preceding and succeeding, as can be seen in figs. 1 and 2. This enlargement is independent of sex, for it is found in both male and female, in sexually immature specimens, and at any time of the year, regardless of the breeding season, which, as recorded by Dr. Van Denburgh, occurs in April. Thus I have noted it in specimens taken on the following dates: February 28, March 17, March 31 (sexually immature), April 17, August 20, October 1 and November 30, and its absence in two specimens taken March 17 and April 17.

At least some others of the urodela show a condition of the tail similar to that of Plethodon. In the collection at the California Academy of Sciences it was present in *Plethodon croceater* and in *Amblystoma opacum*. The former species occurs in California, the latter east of the Mississippi, from Massachusetts to Louisiana, so the peculiarity is not a matter of geographic range.

An examination of the tail, both macroscopic and microscopic, reveals the anatomical nature of the swelling. The dorsal half of the epidermis of this organ is covered with minute and thickly crowded pores which can be seen with the naked eye. These are present alike in tails enlarged and in those that are not, as shown in fig. 1. The skin of this region is enormously thickened.

Even in the unswollen tail it measures about one-sixth of the vertical diameter of the organ, but in the swollen ones it may reach one-fourth of this dimension, as in the one shown in fig. 4. The thickness decreases from a point just outside the median dorsal line, and becomes of ordinary depth at the meeting of the dark upper and light lower surfaces. From the caudal border of the anus to within nearly a millimeter of the tip this thickened skin sits like a saddle astride the tail's back.

A median dorsal groove occurs on the inner surface of this skin. The whole of the surface exhibits a marked granular appearance to the unaided eye, which is due, as can be seen with a hand lens, to the presence of distinct bodies so closely crowded together that they become five-, six- and seven-sided in outline. On cross section of the skin these bodies appear as columns about four times higher than wide, flattened at the inner ends, the spaces between the outer ends being filled with smaller cylindrical and spherical bodies, fig. 4, Pl. XVI. These structures prove to be greatly enlarged epidermal glands. Each one is made up of large granular cells, and opens to the exterior by a short duct. The smaller cylindrical and spherical bodies mentioned in the preceding paragraph are also glands. As will be seen in fig. 4, the large glands on the dorsal side of the tail grade into the small ones which occur on the ventral side of that organ, and, less thickly crowded on almost every other portion of the body of the animal. In the swollen tail they are closely set together and filled to the utmost with secretion, while in the unswollen one, as shown in fig. 5, they have discharged and new glands are forming.

In neither Diemyctylus nor Batrachoseps do we find any such development of tail glands. The skin everywhere of these species is richly provided with glands, and in Diemyctylus these are large and abundant in certain regions of the back, but they are not massed upon the tail.

It is evident from a comparison of the tail glands of Plethodon, both with those from other regions of the body of the same animal and with those of other species, that we are dealing here with structures widely distributed among the Batrachia. Numerous authors have described them in many species of both urodela and anura. Their massing along the ridge of the tail is not uncommon

in Amblystoma and Chondrotus, for Cope describes it in *A. punctatum*, *conspersum*, *opacum*, *talpoideum* and *copeanum*, *Chondrotus paroticus* and *decorticatus*, and at least in *A. opacum* the tail is swollen in consequence. Cope does not mention this condition in *Plethodon oregonensis* nor in *P. croceater*, though he does describe it in *P. glutinosus*.

The glands of Plethodon secrete a milky fluid which is poured out freely when the animal is stimulated by an induction current, either upon the back or upon the tail. In the same way they respond to the touch of a drop of acid, to irritation in the form of stroking with a knife blade, to a forcible holding of the tail either in the hand or in the jaws of a snake. That the secretion is acid is shown by its turning blue litmus paper red. It appears not to be mucus, for it is insoluble in water, or in water to which has been added ammonia or caustic potash or salt, whether the solutions are strong or dilute, cold or boiling hot. The glands upon the tail, as well as those from other regions of the body, likewise fail to respond to specific stains for mucus. Thionin, used by Nicoglu to discriminate glands of different character in various European Tritons, stains the sublingual of the cat, the œsophagus of Plethodon and the skin of the earthworm in three minutes, so that the mucus stands out in red violet upon the blue background of the rest of the cell. Mayer's mucicarmine also in fifteen minutes brings out the mucus in the cat's sublingual red against a pink background. None of the skin glands of Plethodon, when treated in these ways, give a mucus reaction. The secretion dissolves at once in a solution of hydrochloric acid. When exposed to the air it quickly hardens into a tough translucent mass. The least trace of it upon the tongue produces a drawing, drying sensation, with an astringent taste. In general the secretion seems to be similar to that of certain glands of other Batrachia, as of *Triton cristatus*, described by Capparelli.

Diemyctylus also, when stimulated electrically, yields a copious milky secretion, not merely upon the tail, but very generally over the whole dorsal surface. Batrachoseps, on the other hand, yielded very little.

Before we take up the question of the significance of these glands, we should consider another phenomenon of quite different

nature presented by two of the species under consideration, namely, the capacity for autotomy of the tail. Batrachoseps drops its tail very readily and on slight provocation, the break occurring at almost any point. It also regenerates this organ. Diemyctylus does not shed its tail. Plethodon stands between Batrachoseps and Diemyctylus in this respect, for though it can and does part with the member, it does this only under stress of the most untoward circumstances. Holding the animal by the tail, irritating it with acid or by an electric current produces no effect. It was not until individuals were put tail foremost, half way down the throat of a snake, that they finally parted with their caudal appendages, and it actually took four encounters with a ring-necked snake to bring off the tail in one specimen. Thus it would appear that Plethodon reserves this act of autotomy as a last resort, using it only when nothing else avails.

As has been said, the break in the tail of Batrachoseps occurs at any point. In Plethodon, on the other hand, so far as direct observation has gone, it comes only at the constriction behind the anus. Thus it took place here in three individuals which shed their tails in the terrarium, in two which were held in the throat of a snake, in one which was attacked by a snake, and it was seen in another which had been regurgitated. It occurred here in an individual which was placed in the killing fluid without a previous anaesthetic, and in numerous museum specimens, probably killed in the same way, the tail showed a weakness in this spot, breaking here very readily. Three specimens from the field were regenerating the organ from this point. In one case only did there seem to be an exception to this rule, and that was in an individual which was regenerating the tail at a point somewhat more than a centimeter from the anus, as shown in fig. 3, Pl. XVI. But this exception is of doubtful significance, for the tail may have suffered some accident or been bitten off, though it seems somewhat inconsistent with the general conclusion to suppose that the animal would permit this loss in preference to parting with the whole organ.

Dissection shows that the division occurs simply by an unlocking of the vertebrae, not by a break in the centrum, as in the case of self-amputation of the lizard's tail.

As might be expected, Plethodon has the power to regenerate the tail. Three specimens found in the field had tails about a centimeter long, white, somewhat translucent and pointed. When autotomy occurs naturally bleeding does not take place. In regenerating, the stump first rounds out with translucent tissue, and then there grows from the middle point a small bud, at first blunt and of uniform diameter afterwards pointed, as shown in fig. 2. It took a month, in an individual whose tail had been amputated, for a tip to grow about four millimeters long, but, as the conditions in this case were artificial, it would not be safe to draw conclusions therefrom as to the rate of regeneration in nature.

We may now return to the question of the physiological significance of the glands. From a comparison of the structure and action of the glands of Plethodon with those of other Batrachia in which the nature of the secretion is known, one is led to suspect that the secretion here is poisonous and protective. Numerous writers have described the product of the various forms of epidermal glands among the Batrachia as milky, acrid, and poisonous. Leydig, for example, as one of the older observers in this field, speaks of it as sharp, irritating, benumbing, and capable of producing death. Capparelli has worked out in great detail the poison of *Triton cristatus*.

In order to test the action of the secretion I made a number of feeding experiments with all three species here treated, the results of which follow.

Batrachoseps is eaten greedily, both by the garter snake, *Thamnophis elegans*, and by the ring-necked snake, *Diadophis amabilis*. At least five tests were made with the Batrachoseps in connection with these two snakes. The taste is perhaps not quite to the snake's liking, for in some cases there was a slight gaping after eating, but in no instance was there the least hesitation in attack. Only once did regurgitation occur and this once it may have been due to over-eating, for the snake had devoured three or four Batrachoseps in quick succession.

Diemyctylus, on the contrary, does not seem to be desirable as food. Out of eleven trials, at periods varying from one hour to a day, an individual of *Thamnophis elegans* only once attacked this

salamander, though the snake was hungry, as was proved by its readily eating Batrachoseps and tadpoles, and though the salamander was by no means too large to discourage an attack. Each time the Diemyctylus was introduced into the terrarium the snake became alert, moved toward the Diemyctylus and apparently made an examination, its nose coming close to the newt's body, its tongue darting out and in. After that the snake withdrew and seldom showed any desire to repeat the test. On the one occasion when the snake did make an attack it had fasted for eleven days. As soon as the Diemyctylus was introduced the Thamnophis made only a hurried examination, then seized the newt by the middle, and, working its jaws from side to side moved up nearly to the head. Then, instead of swallowing its captive, the snake slowly relinquished its hold and finally dropped its intended victim. That this foretaste of its anticipated meal was enough to satisfy the Thamnophis seemed clear, for it went about for an hour afterwards, opening its jaws very wide at frequent intervals, as if trying to get rid of a bad taste; and the lesson was learned so thoroughly that in the three remaining trials it took no notice of the newt.

With Plethodon the tests have been most instructive in connection with the ring-necked snake, *Diadophis amabilis*. This is a favorable subject for experiment, for, its haunts being the same as those of the salamander, it is no doubt a natural enemy. I had made numerous trials with the Plethodon, before finding a Diadophis, such as forcing a frog and a garter snake to swallow either the tail or the entire animal. On one occasion a garter snake, left with two small Plethodons, devoured both, but these specimens were both small and without tails, consequently could be eaten with impunity. In every case of forced feeding both frog and snake went through the act of gagging after eating, and one snake, after three days, regurgitated the whole salamander with only a portion of the head digested. The same frog ate a piece of raw meat of equal size and voluntarily took a number of tiny toads without gaping. On the other hand it is not safe to say that the gagging was in every case due to a bad taste or that the regurgitation was the result of disagreeable or poisonous qualities. The violent methods of feeding in these

experiments made the interpretation of the results doubtful, and, besides, did not fully answer the question whether *under natural conditions* the salamander is really protected by its poison.

After having tried the Diadophis, to see of it was hungry, by giving it a Batrachoseps, I waited a day and then introduced a swollen-tailed Plethodon into the terrarium with the snake. The snake was hungry and a series of encounters ensued. Three times the Diadophis had the Plethodon by the neck and would surely have disposed of it had I not beaten off the snake in order to give the Plethodon every chance to save itself. Three times the Diadophis seized the Plethodon round the middle and worked toward the caudal end of the body. Then each time, but not until then, the salamander poured out the milky secretion on its tail and the snake released its hold. Upon the fourth attack of this kind the Plethodon dropped its tail and wriggled away, only to lose the battle, for then the Diadophis devoured it tail and all, not however without some gagging afterwards.

The next morning, the Diadophis being in the same condition of hunger as on the day before, I put with it another Plethodon. As it crawled up upon the snake's coils, the latter became aroused and glided toward the salamander, its tongue darting out and in. The Plethodon, which had been perfectly motionless for some time, suddenly, without moving the rest of the body, raised its tail and with a *sidewise motion* struck the Diadophis squarely in the face. Somewhat daunted by this reception the enemy retreated, but in a moment or two came up again, this time from behind. When the snake's mouth was very near, the salamander, rigid in every other part of its body, suddenly raised its tail, as a cat arches its back at a worrying dog. At the same time the tail became covered with the milky fluid. Diadophis, making a brief survey, retreated again, and the Plethodon, after remaining motionless in this position for several minutes, long enough for me to make a sketch from which fig. 6, Pl. XVI, was drawn, gradually lowered its tail until it rested on the floor. The snake did not approach the salamander again.

It would be rash in most instances to draw conclusions from a single case or a single experiment, but there may be times when that single case or that one experiment is sufficient to determine

the point at issue. From this experiment, only one, it is true, but crucial, I judge that the tail glands in this species offer a partial protection to the animal. They may, perhaps by some offensive odor or by come irritating volatile product, ward off an enemy at times. But this means of protection is only partial, as shown by the experiments, for at times not even the taste of the secretion prevents the animal's destruction.

To summarize the results of the experiments: We have, in these three species, a graduated series so far as the relation of the power of autotomy and the presence of these poison glands are concerned. Batrachoseps yields comparatively little poisonous secretion when stimulated; Plethodon yields it abundantly on the tail and Diemyctylus pours it out very generally over the dorsal surface of the body. Batrachoseps is eaten with avidity by snakes. Plethodon is not rejected, but Diemyctylus seems not to be taken at all as food. In Batrachoseps, where the secretion is slight, autotomy occurs on *little provocation and at almost any point*. In Plethodon, where the secretion is restricted to the tail though abundant there, autotomy occurs only as a last desperate resource and but in one region. In Diemyctylus where the secretion is copious and general over the body autotomy does not take place.

Finally, passing from the region of fact and entering that of hypothesis, it seems fair to conclude that we have in these three species a case of adaptive correlation between autotomy and protective secretion. Batrachoseps appears to have, in its great tail-shedding power, some compensation for its limited defensive glands. Diemyctylus has no need of this, being sufficiently safe, so far as one means of defense is concerned, in its own abundant secretion. And, finally, it seems probable that when its tail secretion fails the Plethodon, this species sheds that organ to supplement the inadequacy of poison.

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PLATE XVI.

Fig. 1.—*Plethodon oregonensis.* From photographs of two alcoholic specimens, showing the extremes of the swollen as compared with unswollen condition of the tail.

Fig. 2.—From a photograph of a living individual in process of regenerating the tail.

Fig. 3.—From a photograph of a living individual in process of regenerating the tail.

Fig. 4.—Cross section of the swollen tail, drawn with the camera lucida. Enlarged 7½ times.
epd.—Epidermis. *l. g.*—Enlarged glands.

Fig. 5.—Cross section of the skin of the unswollen tail, drawn with the camera lucida under a low magnification.
d. g.—Discharged glands. *n. g.*—Newly-formed glands.

Fig. 6.—Drawing finished from a pencil sketch. This shows the Plethodon in the act of defending itself against *Diadophis amabilis.*

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REGENERATION AND NON-SEXUAL REPRODUCTION IN SAGARTIA DAVISI

BY

HARRY BEAL TORREY AND JANET RUTH MERY

This paper embodies a preliminary account of investigations, as yet unfinished, the present results of which it has seemed desirable to publish without waiting for the completion of our work.

I. NON-SEXUAL REPRODUCTION IN SAGARTIA DAVISI.

S. davisi reproduces non-sexually by longitudinal fission only, though in some cases the fission resembles rather closely in some respects the process of basal fragmentation so common in *Metridium* and, according to G. C. Davenport ('03), probably occurring in *S. luciae*, the eastern representative of *S. davisi*. Three types of fission are distinguishable in *S. davisi:*

1. Aboral-oral fission by constriction, accompanied by rupture.

2. Aboral-oral fission by constriction alone.

3. Fission proceeding from side to side, by rupture.

McCrady ('58) observed aboral-oral fission in a South Carolina cribrinid which he called *Actinia cavernosa,* but he did not see the completion of the process. G. C. Davenport has recently ('00, '03) observed similar phenomena in *S. luciae*, following the process to completion. In the descriptions of both authors few details are mentioned, and no distinction is made between fission by constriction and fission by rupture. Carlgren ('93) has recorded a case of aboral-oral fission in *Protanthea simplex;* but, as Torrey ('98) indicated in commenting on a

similar case in *Metridium*, such exceptions may be due to accidental rupture of the foot disk rather than to a normal fission process.

The third type of fission enumerated was observed by Mrs. Thynne ('59), in what she asserted to be a species of caryophyllian coral, though it is questionable whether the corals were not really anemones. According to her account, the eggs of *Cyathina smithi*, laid in her aquarium, produced polyps which grew to adult size without forming skeletons. It was among such individuals that she obtained the following facts regarding their non-sexual reproduction. The mouth expands, and the polyp assumes a rectangular shape; the body wall, oesophagus, mouth and foot disk between any two adjacent corners break down; the same thing then occurs between the other two corners, dividing the mother into two portions. Each of the latter ordinarily divides again, so that ultimately four pieces, corresponding to the four corners of the rectangle, are isolated and become perfect by regeneration. Occasionally but two or three polyps arise from one in this way. *S. davisi* reproduces similarly; *S. luciae* will probably be found to be in the same category.

In every process of reproduction by fission, a period of destruction (fission) can be more or less clearly distinguished from a period of construction (regeneration). In describing the methods of non-sexual reproduction which are associated with the three varieties of longitudinal fission in *S. davisi*, it will be convenient to make use of this distinction.

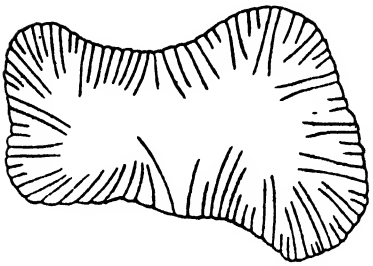


Fig. 1. The elongated foot disk in an early stage of division, the mesenteries arranged in two systems. From below.

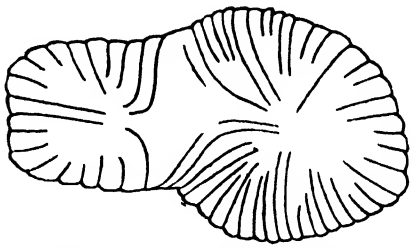


Fig. 2. Foot disk of dividing polyp from below. Tension indicated by course of mesenteries.

1. The first method to be considered may be characterized as aboral-oral fission by constriction and rupture, with subsequent repair.

(*a*) Fission. The first signs of division appear in the rearrangement of the mesenteries on the semi-transparent foot disk, and the elongation of the latter along a line parellel with the major axis of the mouth by the active locomotion of two opposite regions away from each other. The typical arrangement of the mesenteries is strictly radial, around a single center. When division begins, the original center gives way to two (Fig. 1), which move farther and farther apart as division progresses.

A glance at Figs. 2 and 3 may make clear what is very apparent in the foot disks from which they were drawn, that the divergence of the centers is accompanied by a tension, which particularly affects the region between them, and is indicated by the course of the mesenteries. The boundary between foot and column, never sharply marked, becomes less and less distinct, especially in the narrowed intermediate region between the incipient foot disks of the future daughter polyps (Fig. 3).

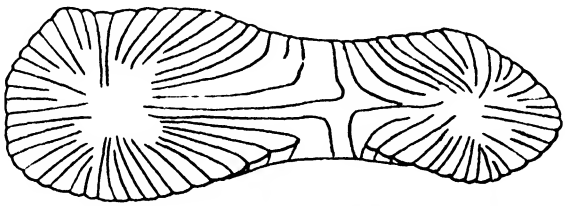


Fig. 3. Foot disk of dividing polyp; a later stage than that shown in Fig. 2. From below.

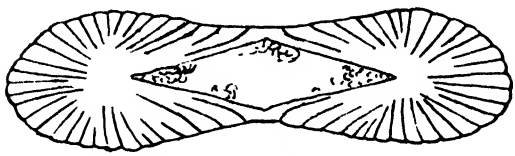


Fig. 4. Foot disk of a dividing polyp, showing rupture. From below.

As a result of the tension, the attenuated tissue on the foot disk between the centers is ruptured before long, and a gaping, diamond-shaped wound is formed (Fig. 4). From this point, the division runs rapidly to completion. The diamond increases

in length, at the same time encroaching in its lesser diameter more and more upon the column, until, with a tear across the mouth disk, the independence of the two moieties is established.

Such, in general, is the process of fission; but there are several facts connected with it which should not be overlooked. The division, which is usually approximately equal, may be very unequal; in rare instances, a polyp is divided into three parts, two large and approximately equal, the third very small. In every case, however, the fission plane passes through the mouth disk, and almost invariably through the mouth also. When the mouth is involved, the fission plane always passes approximately perpendicular to its major axis. If the dividing polyp be diglyphic, the division (into two) gives one siphonoglyph to each portion.* It has been frequently observed that polyps resulting from fission themselves divide, and in every case the second fission plane parallels the first, that is, it also passes perpendicular to the major axis of the mouth. The second division may succeed the first before the regeneration of a second siphonoglyph, as sections show, so that not only may division occur in monoglyphic polyps, but in such cases, may give rise to polyps which have no siphonogyphs at first. Rearrangements of mesenteries foreshadowing both first and second divisions may occur together in the undivided polyp, in rare cases.

With respect to the relation of the fission plane to the mesenteries, it can be said that among 51 polyps resulting from fission, sections taken before new mesenteries had had time to regenerate and complicate the investigation, showed that ten had resulted from division through exocoels, thirty-two from division through endocoels (in a large but unrecorded majority of cases, between mesenteries which reached the oesophagus), and nine from division through an exocoel on one side and an endocoel on the other.

The rate of fission varies within rather wide limits. The process may begin and end within twenty-four hours, as in *S. luciae* also (Davenport, '03), or it may require weeks for completion. Experiments indicate that the food supply may be

*Cf. M. dianthus (Torrey, '98), in which species the fission plane is parallel to the greater axis of the mouth, and divides the one siphonoglyph in monoglyphic, one or both in diglyphic polyps.

a factor in the result. Davenport has reported that "by feeding to repletion, division already begun could be delayed, even apparently prevented," in *S. luciae*. Our own experiments pointed in a similar direction, but were not conclusive. There is no question that when food in the shape of a small amphipod or morsel of flesh is seized by a dividing polyp, the process of division ceases for a time; the tension in the elongated foot decreases, the centers of the mesenterial systems draw together while remaining quite distinct, and do not move apart until the food is digested and disposed of. But similar delay may be caused by strong mechanical stimulation at short intervals. And it is questionable whether it is the mechanical or chemical stimulation of the tissues of the body by the food, or their abundant nourishment by absorption of the products of digestion, that is at the root of the matter. The fact that aquaria polyps which show the effects of starvation for long periods by actually decreasing in size, do not appear to divide, gives some countenance to the former view.

(*b*) The regenerative processes succeeding fission of this type are not sufficiently distinct from those succeeding those of the second type to warrant a separate description. For this reason they will be described after fission of the second type has been considered.

2. The second method of non-sexual reproduction in *S. davisi* to be considered resembles the process described by Mrs. Thynne.

(*a*) Fission is not preceded by a rearrangement of mesenteries about two centers, and is usually completed within twenty-four, often within fifteen hours (i.e., over night). It may result in the formation of two, three, four or five independent pieces which may be equal in size but are usually unequal, especially when there are more than two. The tear begins on one side, involving all tissues from column wall to oesophagus inclusive. Meanwhile, the moieties separate as in fission of the first type, and the tissues on the other side of the body between the two are put upon the stretch. The prompt completion of the division leads ordinarily to but two individuals, the tear proceeding in general perpendicularly to the major mouth axis. It occasion-

ally happens, however, that before the division is completed, an area of the foot disk near one of the free edges produced by the tear, becomes secondarily attached and ceases to follow the migrations of the moiety with which it is connected. A new strain in the intermediate tissue results, ending in complete rupture and the establishment, by regeneration, of a third polyp, usually much smaller than the other two, but possessing from the first a portion of the oesophagus, mouth disk and a few tentacles. A fourth and rarely a fifth fragment may be formed similarly before the division may be said to have given way to a period of repair. In the last case, the fission plane passes quite irregularly with respect to the original major mouth axis. The process as a whole is strikingly irregular, and appears to differ from the basal fragmentation of *Metridium* only in so far as each fragment retains a bit of the oesophagus and a few tentacles.

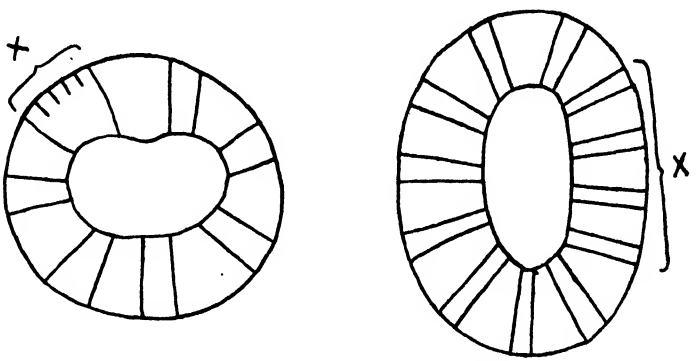
(*b*) Regeneration succeeding fission of both foregoing types. As soon as fission has been accomplished, the torn edges of the body wall roll in and the wound closes, with the tentacles retracted. In fission of the second type, the edges begin to roll in on one side as soon as formed, without waiting for the completion of the division on the other side. In a day or two, each new polyp now expands, and the edges of the wound may be seen to have fused. Along the line of fusion a strip of new tissue begins to appear, easily recognizable by its color, which is many shades lighter than the rest of the body wall. This is the zone of regeneration, in which new tentacles and mesenteries soon make their appearance.

The mesenteries are the first to develop, but there is no constant relation between the appearance of mesenteries and tentacles, the latter appearing now earlier, now later, and in no absolutely fixed order. The first pair of mesenteries arises in the middle of the zone, and is soon followed by two other mesenteries, one on each side of the original pair. This stage with four mesenteries of approximately equal size is so frequently met with that it was some time before it was discovered that they do not appear simultaneously. Next, stages with six mesenteries are obtained, due probably to the addition of a mesentery on each side of the first four. But beyond this point we can say

nothing definite as to the order of their appearance. The first pair, second pair, or all of these first six mesenteries, and indeed of the first eight or ten mesenteries, may reach the oesophagus. There is no fixed order of increase in size.

The first tentacle appears between the first two mesenteries. Two tentacles follow simultaneously, one on each side of the group of the first four. Then four tentacles appear, not always simultaneously, however, one on each side of each of the last two. Beyond this point the regeneration of tentacles was not followed.

We have been unable as yet to ascertain definitely whether the process of regeneration results in bringing the polyp back to its original condition as regards number and arrangement of mesenteries and tentacles; or, to state the question in a different form, whether the number and arrangement of new tentacles and mesenteries are in any way conditioned by the number of old tentacles and mesenteries at the beginning of the regeneration. These problems will admit of ready solution as soon as a further supply of materials is obtained. It may be definitely said, however, that regeneration does not tend to restore a particular structural type. The sexual type, at present unknown, is probably itself variable. A small percentage of regular hexamerous diglyphic polyps is found. If this be assumed as the sexual type, which will then be the fundamental type of the species, in all probability, it is clear that such regeneration as shown in Figs. 5 and 6 does not tend to establish it. Many polyps are



Figs. 5 and 6. Semi-diagrammatic cross sections of polyps in process of regeneration, showing perfect mesenteries and zone of regeneration (X).

met with also which show no signs of a zone of regeneration, but possess only two pairs of perfect mesenteries. If they are products of fission, as is probable, then in them regeneration seems to be at a standstill. Such cases suggest the influence of external factors; lack of food, for instance, might alone prevent the return to the parent condition which might otherwise have occurred.

3. The third method of non-sexual reproduction in *S. davisi* may be described as aboral-oral division by constriction. This is the least conspicuous method of the three, occurring so rarely that we have never seen the completion of the process in a *normal* individual. In consequence, we cannot demonstrate its normal occurrence, but are strongly inclined, from indirect evidence, to believe that it does actually play some part, though a very small one, in the propagation of the species.

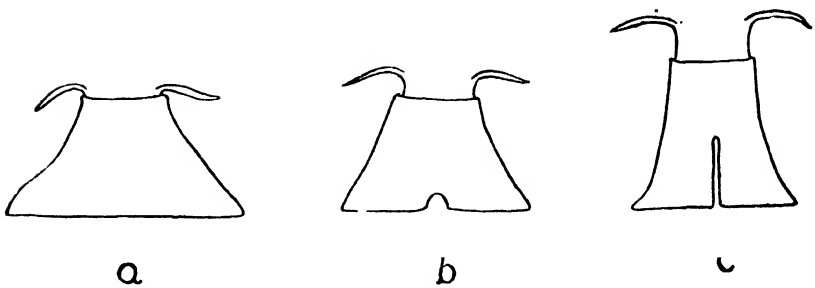


Fig. 7, a, b, c. A series of polyps which may represent different stages aboral-oral fission by constriction.

In the first place, cases that appear to represent stages in the process have been found which can be arranged in a progressive series (Fig. 7, *a, b, c*). Case *c* might have risen as the result of an accidental tear through the foot disk, a condition we have mentioned as sometimes occurring in *Metridium*, where it has no connection with normal methods of non-sexual reproduction. Against this view, results of experiments to be described below may be brought, indicating that a tear of such proportions would probably initiate a fission that would reach a speedy completion. We have no facts to indicate that *c* represents a double monster sprung from an abnormal embryo, and do not favor such a view.

On the other hand, the condition represented in *a* has been met with many times as a *resting* condition, though identical

with that early stage of fission of the first type which immediately preceded a tear (Figs. 2, 3). The condition represented in *b* may be readily derived from *a*, and there is evidence that it is so derived. For the condition represented by *b* has been seen to merge into the condition represented by *a* as a result of a separation of the two foot disks and a consequent stretching of the intermediate tissue. We think it highly probable that during a period of comparative inactivity in such a case as *a*, two foot disks have been differentiated from the tissue of the isthmus connecting them, this isthmus being formed largely if not exclusively by tissue of the body wall (cf. Fig. 3).

The best evidence, however, is to be obtained from the actual division of one polyp, abnormal, it is true, but doubly interesting on that account. This polyp was abnormal in that it possessed a second mouth and set of tentacles on the side of the column. It was unique in this respect among the many hundreds of polyps we have examined; and since budding is unknown in the species, we are disposed to believe that the supernumerary structures were produced as the result of a wound on the column; that they can be so produced experimentally will be shown later.

When the abnormal polyp was first observed, no signs of division were noticed in the foot disk. A few days later the mesenteries on the foot disk were seen to be arranged around two centers. The foot disk had lengthened along the line passing through both centers. Two foot disks were soon distinguishable, separated by a constriction which proceeded slowly upward. Without sign of rupture, a complete division was finally effected. Instead of passing as usual across the mouth disk, however, the fission plane passed *between the two mouth disks*, a peculiarity for which the presence of the supernumerary mouth and tentacles must be in some way accountable. The direction of the fission plane with respect to the major axis of either mouth was not observed, so that it still remained to be determined whether or not the doubling of the mouth disks, besides modifying to some extent the direction taken by the fission plane, might not also have precipitated the division. By way of solution, wounds were made in the columns of a number of polyps in whose foot disks there were no signs of division.

Simple cuts and punctures healed readily without the production of new structures. When pieces were cut out of the body wall, and the fusion of the edges of the wound were thus hindered, better results were obtained—six double-headed polyps in all. None of these showed any tendency to divide in any way, though they were watched for three weeks. This result looks like a demonstration of the view that division is not initiated by a doubling of mouth and mouth disk, and is consequently little less than a demonstration of the normal occurrence of fission by constriction in *S. davisi*. We shall repeat the experiments on a larger scale.

II. CAUSES OF FISSION.

Fission of the first two types in *S. davisi* depends to such a degree upon active movements of different areas of the foot disk in opposite directions that the idea readily suggests itself that the establishment of some sort of physiological discontinuity between these areas may be the key to the causal problem. A solution was attempted by experimentation.

Two sets of experiments produced slightly varying results.

In the first set, twelve polyps were cut from foot half way to mouth, the cut being perpendicular to the major mouth axis (*i.e.*, parallel with the course of a normal fission plane); one had divided in six days, two more in twelve days. Eight polyps were cut from mouth half way to foot, also perpendicular to major mouth axis; two had divided in three days. In the second set, eight polyps were cut from mouth half way to foot, parallel with major mouth axis. In one of these, the wound was repeatedly reopened, but healed again in every case, and no division resulted. Three polyps were cut half way to foot disk, across the major mouth axis; no division resulted.

Six polyps were cut from foot half way to mouth, across major mouth axis; in twenty-four hours three had divided. It was found that if a polyp which is beginning to divide be cut parallel with major mouth axis half way to the foot, the division is inhibited until the wound is healed, and if the latter is reopened, as was done repeatedly in one case, the division takes place only after the wound has finally closed.

The difference between the two sets of experiments lies in the facts that according to the first set, 25 per cent of the polyps cut from the mouth toward the foot disk, across the mouth, divided as against 25 per cent of those cut from the foot toward the mouth, perpendicular to the major mouth axis, and they divided more rapidly; while according to the second set, *none* of the polyps cut across the mouth toward the foot disk divided, although *50 per cent* of those cut from foot toward mouth, perpendicular to the major mouth axis, did divide. This discrepancy may disappear with farther experimentation on larger numbers of polyps and with especial care to keep the wounds open.

It appears to be clear, however, from these experiments, that an interruption of the physical continuity of two portions of a polyp by means of a cut parallel with the course which would be taken by a normal fission plane, tends to interfere with the physiological interaction of the separated regions and initiate the process of fission. This is especially true when the cut follows the aboral-oral course of the normal fission plane (second set of experiments).

Double structures have been produced in various animals by similar experiments: in Hydra notably by Trembley, in planarians by Duges, Morgan, Van Duyne and others, in lizards by Tornier. The partial separation of the first two cells of the sea urchin (Driesch) and Amphioxus (Wilson) leads to even more marked results.* In all of these cases, normal physiological connections have been broken; Morgan is disposed to believe that these physiological connections are in the shape of some sort of tension. The doubling of parts, however, never involves the entire body; there is no evidence of a stimulus to division. Perhaps this is because division of the types made possible by the experiments does not occur normally in any of the species concerned. Yet in *Corymorpha palma,* separation of the two individuals developed heteromorphically on the opposite ends of a fragment of stem has been observed; the discontinuity between the two ends shown by the development of two hydranths was further emphasized by the subsequent division, which never occurs under normal conditions.

*See Morgan ('01), for an account of these cases and the literature of the subject.

But while discontinuity of some sort is probably at the basis of the phenomenon of fission in *S. davisi*, it is apparent that the experiments go no farther than to point out this fact. Why the mesenteries in an unharmed polyp begin to group themselves about two centers, and why opposite areas in the foot disk move away from each other constantly only in polyps which are to divide, are problems which still await solution. A possible explanation of the *direction* of the fission plane may be suggested, however. This plane passes perpendicular to the line of greatest strain. This line of strain is parallel with the major mouth axis, and at the ends of the mouth lie the directive mesenteries. There can be no question that the arrangement of the muscle bands on the outer sides of the directives and near the oesophagus leads to mechanical results which are different from those achieved by all the other muscle bands, which lie on the inner sides of the non-directive mesenteries and farther from the oesophagus. This mechanical difference is always correlated with the shape of the mouth and may be sufficient to determine the direction of the line of greatest strain and consequently the direction of the fission plane in a dividing polyp. The uniform failure to divide of polyps which were cut perpendicular to the direction of the normal fission plane lends support to this view.

III. HETEROMORPHOSIS.

Until the last few years heteromorphosis has been quite unknown among the *Anthozoa*. A typical example was recently obtained by Wilson ('03) in Renilla, a new hydranth regenerating on the aboral end of an extirpated avial polyp of a young colony. Hazen ('02), in discussing the factors which determine the orientation of regenerating pieces of *S. luciae*, says that a pedal disk is produced at the point of contact with the substratum, no matter how the piece falls, provided it is not subsequently disturbed. There is no specific statement that a pedal disk was ever regenerated at the oral end of a piece, and the brevity of the account leaves this in doubt. There appear to be no published observations on the appearance of a hydranth on the aboral end of a regenerating anemone, though attempts have been made on several species, notably by Loeb, to bring about

this result. *S. davisi* offers no difficulties in this direction; more than 50 per cent of the anemones operated upon gave positive results. The facts obtained up to this time are given below.

Twenty polyps were divided by transverse cuts into oral portions which included mouth and tentacles, and aboral portions which included the foot disk. Nine were cut through the capitulum, so that the oral portions were very short. Eleven were cut so that the oral portions were each half the length of the original column.

Of the nine shorter pieces, four rested with mouth disk upward, five with aboral end upward; two of the former, but none of the latter, had developed hydranths in five weeks.

Of the eleven longer pieces, four rested with mouth disk upward, seven with aboral end upward; in less than four weeks all of the former had developed aboral hydranths, while of the latter five had developed aboral hydranths, one had produced both foot and hydranth aborally and one was a normal polyp.

With respect to the factors involved in these results, Hazen has suggested that the position of the axis in regenerating pieces of *S. luciae* might be determined by a geotropic influence or by a combination of geotropism and stereotropism; the foot disk being formed at the point of contact (itself determined by gravity), the hydranth at the opposite (upper) end. This suggestion hardly fits the facts of heteromorphosis which have been enumerated. Gravity cannot determine the presence or absence of an aboral hydranth when the latter develops regardless of the orientation of the piece. We cannot speak so surely about the factor of contact. It is possible that the aboral cut surfaces of the pieces in our experiments which are resting mouth up, did not touch the substratum for a sufficient length of time to determine the development of a foot disk; they certainly did not adhere. On the other hand, it is odd that the only foot disks developing on the aboral ends of pieces, appeared on two longer pieces, both of which rested on their mouth disks, not on the aboral cut surfaces. Internal factors seem to have been more potent than external in this case. It is probable, however, that future experiments will show a certain influence of contact upon the development of the foot disk in regenerating pieces to

S. davisi, as Hazen has already shown it for *S. luciae*. We have obtained, so far, one case which supports this view. The aboral portions of the polyps used in the previous experiments were inverted, so that their oral cut surfaces came in contact with the substratum. In every case but one the pieces righted themselves and regeneration of hydranths ensued. The single exception remained as it was placed and developed over the cut end a smooth surface which resembled a foot in appearance, though it did not adhere. The piece finally died.

Two other factors should be noticed: size of piece and region of cut. The longer pieces developed heteromorphically much more readily than the shorter ones. We have not been able as yet, owing to difficulties of manipulation, to compare pieces of similar length from different regions of the column to determine directly the relative value of the two factors. It is highly probable, however, that size is the more important of the two, since the shorter pieces produced neither foot disks nor hydranths in 75 per cent of the cases, indicating a regenerative capacity in general inferior to that of the longer pieces.

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THE STRUCTURE AND REGENERATION OF THE POISON GLANDS OF PLETHODON.

BY

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It has long since been held that the skin glands of both the Urodela and the Anura are of two kinds. This distinction was first made by Ascherson '40 in an investigation of the glands in the web of live frogs, and was based upon the size, shape, and location of the glands without regard to function or microscopic structure. That the skin of Amphibians secretes a substance other than the well-known mucus, and clearly poisonous, has been shown by many physiological and toxicological experiments and investigations (Albini '56; Boulenger '92; Calmels '83; Capparrelli '83; Dutarte '89; Gratiolet and Cloez '51–'52; Hubbard '03; Phisalix-Picot '00), and the facts gained from experiment are upheld as far as possible by histological evidence. Microscopic examination shows that there is more than one kind of gland. (Ancel '02; Coghill '99; Eberth '69; Eckhard '49; Engelmann '72; Hensche '56; Leydig '76 *a*; Paulicki '85; Phisalix-Picot '00; Schultz 89; Seeck '91; Stieda '65; Szczesny '67; Wiedersheim '86). These have generally been distinguished as granular (Körnerdrüsen) and clear, according to the appearance of the secretion contained in them, the former having been almost unanimously looked upon as making the poison series, the latter the mucous series. The suggestion has been made, however, that the various glands are only the young and old stages of one sort of gland (Junius '98), and this question will receive further consideration in the present paper.

The poison glands are in most cases much larger than those of the mucous variety, and their enormous cells (Riesenzellen of Leydig) completely fill the interior of the gland so that there is no lumen. This character distinguishes these glands from the others, which are provided with a low, cubical epithelium surrounding a capacious lumen. (Pl. XX, Fig. 2.) The mucous secretion filling the lumen is very distinct from the heavily granular contents of the cells of the poison glands. (Pl. XXIII, Fig. 31.) The two sorts of glands are further distinguished by other features, chief of which is the staining reaction of the mucous secretion (Nicoglu '93; Hoyer '90). These observers used thionin as a specific stain for mucus and found that the small glands stain rose-red while the others are uncolored.

The foregoing general facts have been determined chiefly upon the Anura and the various European Salamanders, especially Triton and Salamandra. But *Plethodon oregonensis*, a salamander found about Berkeley, forms a particularly interesting object for the study of the poison glands because of their unusual development on the tail of this animal. This seems to be a protective character associated to some extent with the ability of the animal to throw off its tail under stress of circumstances. It has been shown by experiment that the secretion of the glands of the tail is poisonous or obnoxious to certain animals, a character which probably belongs to the dorsal glands (Hubbard, '03), which are very large and much more developed than elsewhere on the body. In this respect Plethodon appears to resemble *Triton cristatus*. (Capparelli '83.) However, the poison glands of Plethodon are not confined to the dorsum of the tail; much smaller ones are found on its ventral surface and also on the trunk and head of the animal, intermingled with mucous glands, which occur in all situations where the poison glands are found.

The principal question considered in the present paper concerns the changes occurring in the formation of the secretion and its expulsion from the glands. In Plethodon this involves the death of the glands, as Seeck ('92), Nicoglu ('93), Vollmer ('93) and others have shown for other Amphibia. The exhausted glands are here renewed or replaced in the manner described by

Heidenhain ('93 *a*), Vollmer ('93) and Nicoglu ('93). This process consists in the growth into the old glands of a new and smaller gland, which, however, is mucus in character, contrary to the statements of Nicoglu ('93), so that the poison glands develop from the mucous to the poison variety. This has been suggested but not definitely shown by Hoyer ('90) and Junius ('98), and distinctly denied by all other investigators of the regeneration of these glands. Under the histological structure of the glands will be considered some new points in the musculature, especially as to the presence in the epidermis of an apparatus for closing and opening the duct. The innervation of the muscles and epithelium of the glands will also receive attention.

This work was done under the direction of Professor C. A. Kofoid, and my heartiest thanks are due him for very kind assistance in every way and for criticism of results.

MATERIAL AND METHODS.

In order to obtain the best insight into the structure of the glands of the tail, sections in three planes have been made of that entire organ. The tissue was in all cases perfectly fresh and was fixed in Zenker's fluid, which has been satisfactory in all respects. Washing in 70% iodine-alcohol followed the use of the fixative.

That bony tissue might not hinder the passage of sections in any plane through the whole tail, the tissue was subsequently decalcified in a 5% aqueous solution of nitric acid for from twelve to twenty-four hours, followed by immersion in a 5% aqueous solution of sodium sulphate for the same length of time, and thorough washing in running water for from twenty-four to forty-eight hours.

Paraffine sections have alone been used, varying in thickness from 3½ to 10 microns. The sections were fixed to the slide by the water-albumen method, and in all cases where possible staining was done on the slide.

A considerable variety of stains has been employed. The most successful have been Mallory's ('00) connective tissue stain (acid fuchsin, phospho-molybdic acid, anilin blue-orange G),

Van Gieson's haemalum and picro-fuchsin, and the iron-haematoxylins of Benda and Heidenhain. I have found it of considerable advantage to increase the percentage of fuchsin in Mallory's stain to as much as 1.5 or 2%. This stain, as a whole, when successful is very beautiful, but its action varies most unaccountably. The staining and differentiation will be perfect in some sections, while in others on the same slide the differential coloration will fail completely. But the range of application of the stain seems to be almost unlimited except for purely cytological work.

Other stains have been used, such as Mayer's neutral and acid haemalums followed by eosin, orange G, erythrosin and Congo red; safranin alone or in combination with light green; ferric chloride haematoxylin, and such special stains as the phosphotungstic acid haematoxylin of Mallory and Cajal's ('03) silver nitrate-pyrogallic acid method for nerves, Tänzer's orcein for elastic fibres, and Mayer's muci-carmine as a mucus stain.

As has been said, the largest poison glands of Plethodon are situated on the back of the tail, and in cross sections (Pl. XX, Fig. 1, *p.gl.*) it may be seen that they lie in that portion of the skin covering the dorsal half of the tail. Here the greatest development is in the glands at either side of the mid-dorsal line, while farther down on the sides they gradually diminish until they are considerably smaller and not readily distinguished by their size from the larger mucous glands. (Pl. XX, Fig. 1, *m.gl.*) The coloration also of the tail gives a clue to the location of the largest glands. The dorsal half of the tail is black or brown, while the ventral half is orange or yellow, and the glands under consideration are confined almost entirely to the darker portion. The mucus glands are found largely on the ventral side of the tail, but they also occur along the dorsal surface. In this region they lie between the necks of the large glands.

The poison glands form large sacs, extending from the epidermis to the inner layer of the corium. (Pl. XX, Fig. 1.) In shape they are elongated, with oval or even somewhat rectangular outline. The ducts are short, and the transition from the body of the gland to the neck and duct is not sharp as in the mucous glands, which are regularly flask-shaped.

It has been shown (Hubbard '03) that the swollen appearance of the tails of some animals is due to the increased development of the poison glands posterior to the well-marked constriction found just behind the cloaca in such cases. That this is really true appears in the study of a series of sections of a swollen tail passing from the tip up to and including part of the cloacal aperture. In the constriction the dorsal glands are very small comparatively, and are here no larger on the back of the tail than on the ventral side. But behind the constriction their development is much greater, and one may trace the regular increase in size as the series passes from the constriction back to the enlarged portion of the tail. Everywhere in the tail, except in the constriction at its base, the difference in size between the glands on the dorsal and ventral surfaces is maintained.

As is well known, the bodies of all the glands lie in a spongy connective tissue, the middle layer of the corium, which in the region of greatest development of the poison glands is increased enormously in thickness (Pl. XX, Fig. 1, *m.c.l.*), being alone from one-sixth to one-fourth or more of the dorsal-ventral dimension of the tail. (Hubbard '03.) The bottoms of the large glands rest upon or come very close to the inner layer of the corium. (Pl. XX, Fig. 1, *i.c.l.*)

The ducts of both mucous and poison glands pass through the outer corium layer and the epidermis, the long axis of the gland which passes through the duct and its mouth being perpendicular to the surface at the point where the duct opens to the exterior.

The histological structures found immediately surrounding the ducts of the poison glands are in no essential points different in Plethodon from those in other salamanders. The funnel cells and their processes (Pl. XX, Figs. 1 and 2, *fl.c.*) are present as in Triton (Nicoglu '93) and in Salamandra (Ancel '02). The membrane-like structure lining the duct belongs to a specialized cell of the epidermis, corresponding to the "stoma cell" of Eberth. As Nicoglu has shown, the mouths of the glands lie within these cells, processes of which extend down in the ducts about as far as the lower limit of the epidermis or a little farther. (Pl. XXIII, Fig. 27, *p.fl.c.*) The prolongations stain black in iron haematoxylin, reddish in Mallory's and yellow in Van Gieson's stain.

In addition to the funnel cells proper, Nicoglu has described the arrangement of the cells in the epidermis which are to replace the funnel cells as they are thrown off at the time of moulting. The same condition is found in Plethodon and does not differ at all from that in Triton (Nicoglu '03) or in Salamandra (Ancel '02). (Pl. XX, Fig. 4; Pl. XXIII, Figs. 27, 28, 29, 30. *rep. c.*)

As further evidence that the cells described as replacement cells really have that function, Plethodon shows that the lower ends of the replacement cells, especially those nearest the duct, extend inward as do the prolongations of the funnel cells. (Pl. XXIII, Fig. 27, *rep. c.*) The arrangement of the former very strongly suggests that they are of the same nature and function as the funnel cells. And in cross sections of the ducts the replacement cells are shown rolled one within the other as in Pl. XX, Fig. 4; Pl. XXI, Fig. 16, *rep. c.* The cell first to replace the one thrown off at moulting immediately surrounds the duct; the cell next to replace this one lies concentrically outside it, and so on. In Mallory's stain the cell boundaries are very distinct, and there can be no doubt of the structure as described either in cross or in longitudinal section of the ducts.

The walls of the gland sacs proper are composed, in many Amphibia, of a number of elements which have been described and all of which need not be discussed at length here. In the most peripheral layer are connective tissue and elastic fibrils, as is shown by the use of Mallory's connective tissse stain for the former and orcein for the latter. Nerves, lying in this layer, also extend over the gland. Inside the connective tissue sheath, as it is generally called, lie the muscle fibres, and next to them the epithelium of the gland.

(See in this connection Drasch '92, '94; Eberth '69; Eckhard '49; Englemann '72; Hensche '56; Leydig '76, *a*, *b*; Paulicki '85; Phisalix-Picot '00; Schuberg '03; Schultz '89; Seeck '91; Stieda '65; Tonkoff '00; Wiedersheim '86.)

Because of the intimate relation between the connective tissues of the gland wall and those of the corium, it is necessary to consider in more detail the structures of the inner, middle and outer layers of the corium. Schuberg ('03) has studied the corium of Axolotl most minutely. I have confirmed his results

in general in Plethodon, and particularly as to the relation of the connective tissue bundles of the inner layer of the corium to those of the middle layer. He found (p. 222) that columns of connective tissue pass perpendicularly from the inner into the middle layer, and seem to serve as mechanical supports for the glands, since under each one such a column of tissue is found. The same arrangement occurs in Plethodon except that the perpendicular bundles do not stand beneath the glands, but around them, as can be seen in longitudinal sections of the glands. (Pl. XX, Fig. 5, *c.t.b.*) In spaces between the large glands or on the ventral side of the tail, the bundles from the inner layer of the corium can be seen especially well. The connective tissue fibres from the wall of the gland unite with the outer layer of the corium which then, lying next the muscle layer of the gland, passes toward the surface of the epidermis and ends on the side of the neck of the gland about a third of the distance between the inner and outer boundaries of the epidermis. (Pl. XX, Fig. 8; Pl. XXIII, Figs. 27, 31.) This appears in both longitudinal and cross sections of the ducts. In the latter can be seen a crescent of connective tissue on each side of the duct between the muscle fibres and one of the replacement cells. (Pl. XXIII, Figs. 28, 29; Pl. XXI, Fig. 16, *c.t.*) Ancel ('02, Pl. IX, Fig. 22) seems to have shown the same in longitudinal section.

The elastic fibres pass through the inner layer of the corium into the middle layer in company with the connective tissue bundles as Schuberg ('03, p. 231) has described. The elastic fibres can be followed around the glands, and over them in tangential sections. The fibres are of nearly the same calibre throughout and all of them take the same general direction, from the inner corium layer perpendicularly or sharply turned toward the outer layer. As in the case with the connective tissue bundles the elastic fibres pass at once around or over the large glands, and are not found arranged perpendicularly beneath them as in Axolotl. (Schuberg '03, p. 232, Fig. 14.) On the surface of the gland they are branched in a few cases; usually, however, only single fibres of wavy, curving and regular outline are visible, ending before the outer corium is reached (Schuberg '03, Pl. XXI, Fig. 9, *el.f.*).

Between the connective tissue layer and the gland epithelium lies the layer of contractile or smooth muscle fibres. These were first shown histologically by Hensche ('56), though before him Ascherson ('40) had observed movements of the living glands. Since this time there has been no doubt of the existence of muscles in the walls of the poison glands (Coghill '99; Drasch '89, '92, '94; Eberth '69; Eckhard '49; Englemann '72; Heidenhain '93 *a*, *b*; Leydig '76, *a*, *b*; Massie '94; Nicoglu '93; Paulicki '85; Phisalix-Picot '00; Schultz '89; Seeck '91; Stieda '65; Szczesny '67; Vollmer '93). As regards the smaller series of glands the question seems to be open. The absence of contractile fibres on them has been used as a character to separate them from the large glands. The muscles of the large glands are arranged in a single layer and have a general meridional direction on the gland, converging toward the upper pole. The fibres are usually simple but may be branched (Pl. XX, Fig. 7); this occurs mostly on the lower part of the gland. Neither do the muscles form a continuous sheet about the gland; the individual fibres are separated by spaces of greater or less extent. I have not been able to find with certainty muscles on glands which are mucous in nature.

The nuclei of the contractile cells, contrary to the description of Nicoglu ('93, p. 437,) and such figures as his and those of Vollmer ('93), lie in the upper region of the glands just outside the uppermost gland cells, yet still well beneath the epidermis (Pl. XX, Fig. 6; Pl. XXIII, Fig. 31, *m.n.*). The first observer mentioned has shown (his Pl. XXII, Fig. 12) the nuclei of the muscle cells in *various locations about the periphery of the glands;* but *in Plethodon the nuclei have a constant position as described and are found only there.* In the region of the nuclei the muscle fibres are considerably larger than elsewhere on the gland, as is shown in Pl. XX, Fig. 8, *m.f.*, or Pl. XXIII, Fig. 31, *m.f.*, so that the muscle, especially in longitudinal sections of the glands, seems to bear a flask-shaped expansion. From this point it is possible to trace a single fibre very nearly to the base of the gland, and also outward around the neck of the gland into the epidermis. (Pl. XX, Fig. 6.) The connection of the muscles with the epidermis has been reported by Nicoglu ('93) and Heidenhain

('93 *b*), and the arrangement in Plethodon is a similar one except as regards the presence of the "Schaltstück" cells described by them. In Plethodon the "Schaltstück" is not demonstrably present except in one or two questionable cases in all my preparations, and Vollmer ('93) found that it is very often absent even in Triton. There can be no doubt, however, that the muscles send processes into the epidermis. This is especially well shown in longitudinal and cross sections of the ducts.

The statement that the muscle nuclei of the poison glands in Plethodon lie only in the necks of the glands, instead of generally distributed about the periphery as held for other animals, may be supported by several facts. In the first place, longitudinal sections of the glands through the duct and mouth show two nuclei, one at each side of the gland where the sac begins to pass over into the duct. In sections of the same plane which pass a little to one side of the duct (Pl. XXI, Fig. 10, *mf.*, *mn.*) may be seen in some cases the obliquely cut ends of as many as seven muscle cells each with its nucleus in situ, and occupying exactly the position relatively of the two lateral nuclei which are shown in the median section. There can be no doubt of their structure.

Cross sections of glands and ducts are also very instructive on this point. In such, especially if stained in Van Gieson (Pl. XX, Fig. 12), there are shown in many cases the light yellow muscle fibers between the gland cells and the connective tissue, when the plane of the section passes more deeply through the gland than the position of the nuclei of the muscles. But when the gland is cut across at the level of the nuclei, the evidence gained from longitudinal sections is even more strikingly upheld. In such cross sections can be seen as many as twelve or fourteen muscle fibres stained light yellow (in Van Gieson), and in very sharp contrast to them the brown or black nuclei. And in this region the section of the muscle is larger than it is deeper in the gland; this corresponds to the flask-shaped enlargement seen in median longitudinal section (Pl. XX, Fig. 8, *mf.*). If a series of frontal or cross sections of the tail is studied, it will be found that while the muscles themselves can be traced until the bottom of the gland is reached, nuclei never appear again which are unmistakably those of the muscle fibres. The only place in

which one can be sure that he is dealing with nuclei of the contractile fibres is in the location above described. Hundreds of sections have been carefully examined and there has never been a case of a fully formed gland in which the muscle nuclei are situated in any position except that described. Not only is this true in stains such as Mallory's and Van Gieson's but also in clear nuclear stains like iron haematoxylin.

That those observers who describe muscle nuclei on the periphery of the gland sacs have mistaken connective tissue nuclei for them, seems to me very probable. Nicoglu ('93, p. 438) says that the nuclei often occupy an eccentric position, so that even with oil immersions one cannot see that there is any protoplasm of the muscle cell about them. His description ('93, p. 436) of the flattened narrow nuclei of the muscle cells applies more to connective tissue nuclei. That these occur in the walls of the glands has been observed by Paulicki ('85, p. 158), who says: "An die Drüsen treten gewöhnlich . . . sich nach oben erstreckend bindegewebige Stränge mit Kernen." And the figures of Schuberg ('03), especially Fig. 28, show that this is true for the glands of Axolotl. From these facts and from my observations on Plethodon it is clear that connective tissue nuclei closely invest the glands, and evidence is added to that already brought forward to show the location of the nuclei of the muscle cells.

The processes of the muscles passing into the epidermis serve to connect the fibres with the outermost layer of the skin. This has been shown, as said before, by Heidenhain ('93) and Nicoglu ('93), as well as by Ancel ('02), and there is nothing to be added to the description given by the former except, as before stated to mention the frequent non-occurrence of the Schaltstück as such. This is a structure described as containing about four cells which are arranged in a ring about the neck of the gland at the lower boundary of the epidermis. The cells form seemingly the principal points of insertion of the muscle fibres, but this cannot be so in Plethodon where the Schaltstück is virtually absent. Otherwise it may simply be said that the upper or outer ends of the muscle fibres pass into the epidermis and end between the replacement cells of the funnel. This can be seen fairly

well in cross sections of the gland ducts in the epidermis where the cut ends of the muscles are seen close beside the funnel cell (Pl. XXIII, Figs. 28, 29, *prol. m.f.*). In good longitudinal sections of the ducts the muscles (Pl. XXIII, Fig. 27, *prol. m.f.*) are seen to end between the older replacement cells which are already elongating into their typical form (same, *rep. c.*). Nicoglu ('93) represents the endings as between the cells, but Ancel ('02) seems to consider them as special parts of cells. At any rate he has shown (Fig. 22) the fibrils as within cells in the epidermis. I have not been able to find such structures as he shows; there can be hardly any doubt that the prolongations of the gland muscles into the epidermis end between the replacement cells. Nicoglu and Heidenhain ('93) and Ancel ('02) have remarked upon the existence of intercellular bridges between the muscle cell on the one hand and ectodermal epithelial cells on the other, as Nicoglu says (p. 440), "von ganz ähnlicher Art wie zwischen den Oberhautzellen selbst."

I have not found the intercellular bridges in Plethodon between epithelial and muscle cells, but all the facts concerning the connection of muscle and epidermal cells have been taken as evidence of the ectodermal origin of the muscles of skin glands. This has been so often commented upon that it is useless to more than call attention to it here. The evidence gained from a study of the development of the glands shows that the muscle fibres come from the Malpighian layer of the epidermis (Ancel '02; Vollmer '93; Junius '98). This, added to the facts already cited, and coupled with the observations of many investigators (Engelmann '72; Seeck '91; Heidenhain '93; Nicoglu '93) seems fairly conclusive that the muscles of the dermal glands are of ectodermal origin: (Compare also in case of sweat glands, v. Kolliker '89; Handbuch des Gewebelehre des Menschen, pp. 138 and 258).

The existence of a sphincter or constrictor muscle for the glands has been claimed by Schultz ('89), who described a band of muscle fibres running around the neck of the gland beneath the meridional layer. This observation has been disproved by Drasch ('94) and Nicoglu ('93), and I have been unable to find such a structure in Plethodon. And there is no evidence of the

epithelial plug of Drasch ('94) for restraining the contents of the gland under pressure. Phisalix-Picot ('00) mentions (pp. 44–45) an orbicular muscle, but gives no description or drawing of it, so that her meaning is obscure. Dilator muscles for the ducts or mouths of the glands have never been described.

However, both dilator and constrictor muscles occur about the mouths of the poison glands of Plethodon. These are best shown in sections of the epidermis parallel to the surface, stained in Mallory's connective tissue stain, which are, of course, also cross sections of the ducts. All three sets of gland muscles may very often be seen in one such section (Pl. XXIII, Fig. 30, *con. m.*, *dil. m.*, *m.f.*; Figs. 28, 29 also). In these cases it will be seen that the duct (*l.d.*) in the epidermis is oval in cross section, and that at each end of the oval is a triangular mass of fibres, staining red in Mallory, as do the muscles on the body of the gland. The fibres converge toward the duct and insert upon the replacement cells nearest the funnel in such a way that by contracting they will bring the lips of the duct together and so close or greatly diminish its lumen (Pl. XXI, Fig. 16). The constrictor fibres are differentiations of the cell whose large nucleus (Pl. XXI, Figs. 14, 16; Pl. XXII, Fig. 28, *nuc. ep. m.c.*), stands at the ends of the elliptical opening of the duct. The fibres lie within this cell as can be especially well seen in longitudinal sections of the glands which do not pass through the duct. Here it appears that the cell of which the constrictor fibres are a part, together with its nucleus, lies in the deepest layer of the epidermis immediately upon the outer layer of the corium. This cell seen in surface view is equal in extent to several of the neighboring epidermal cells, but in cross section it is very much flattened (Pl. XXI, Fig. 13). Ancel ('02) has figured such a cell, but gives no clue as to its function.

The dilator fibres belong to the same cell of which the constrictors form a part, and are at a slightly lower level seemingly than the latter. The action of the dilator is two fold. Some fibres pass around the ends of the oval opening of the duct (Pl. XXIII, Fig. 28, *dil. m.*; Pl. XXI, Fig. 14) and when they shorten they tend to separate the lips of the lumen more widely, by pressing at the ends of the ellipse. This is evident when it

is seen that the mass of dilators is often concave in outline toward the center of the duct (Pl. XXIII, Fig. 28; Pl. XXI, Fig. 15, *dil. m.*), so that in contraction the fibres first mentioned pull in the general direction of the major axis and toward the center of the ellipse. Other dilator fibres attach at the edges of the duct near the end (Pl. XXI, Fig. 14), and in shortening pull in the direction of the minor axis of the ellipse, thus widening the lumen by spreading its walls at the tips of the oval. (Pl. XXI, Fig. 14.) The entire effect of the dilator fibres is to make the aperture of the duct nearly circular, thus offering freer exit to the secretion. Their action would be to open the duct from the form shown in Pl. XXI, Fig. 16, to that, for example, in Pl. XXIII, Fig. 30.

The fact that the constrictor and dilator fibres lie entirely within the epidermis need not militate against their having the function of muscles, for in the case of the intrinsic gland musculature it has been well established that it has an ectodermal origin. It is certain that the arrangement and appearance of the fibres described as constrictor and dilator muscles are such as to suggest very strongly both that nature and function. The coloration in Mallory is exactly that of the smooth muscles of the glands; and the convergence of the constrictor fibres to their insertion in a position where contraction would close the duct; the endings of the dilators in places to be of greatest advantage in widening it when the muscles contract—all these facts lead one to conclude that he has to deal with an apparatus for closing and opening the ducts of the glands.

The muscles of the poison glands, as has been said, immediately envelop the secretory cells. The entire gland is filled with enormous cells, the generally recognized "Giftzellen" of many authors or the "giant cells" of Leydig. In such glands a lumen does not exist; this is especially well shown in sections of the tail of a tadpole 38 mm. in length, in which the cell boundaries are distinct, the secretion not yet being present in sufficient quantities to obliterate them. There it will be seen that the ends of the cells are in contact with the middle of the gland, thus doing away with any trace of a lumen (Nicoglu '93; Seeck '91; Calmels '83). A glance at the figures will serve to distinguish

in this respect the poison and mucous glands; the latter have capacious lumens (Pl. XX, Fig. 2), often filled with a clear secretion. The large gland cells each have a number of nuclei (Nicoglu '93; Drasch '92), not over four in Plethodon. They are round or oval, of regular outline, and lie normally upon or very near the wall of the gland, and so at the base of the cells. The internal structure of the nuclei is simple. There is a scanty network and few chromatin granules; usually also one or two nucleoli.

The cells and nuclei of the small or mucous glands are distinct in every way from those of the poison glands. The cells are low and cubical and show a filar structure (pseudo-filar, Nicoglu '93). This is seen in sections stained either with Van Gieson, Mallory or iron haematoxylin. The nuclei are smaller than those of the poison glands, and angular instead of regular in outline. They invariably stain intensely black in iron haematoxylin, remaining so when the nuclei of the giant cells have decolorized to a very faint gray (Pl. XX, Figs. 2, 3; Pl. XXII, Figs. 18, 19, 20).

A general comparison of the two sorts of glands might be instituted in some such terms as these. The poison glands are very much larger than the mucous glands, and have contractile walls; the mucous glands lack this character. The extreme dimensions of the former on the tail are approximately from 1400 microns in length and 380 microns in breadth to 680 microns in length by 200 microns in breadth, and half the latter figures on the body. The mucus glands vary from 93 by 90 microns on the tail to 60 by 40 microns on the body. This alone, without closer inspection, would serve to generally distinguish the two varieties of glands; but in addition the poison glands have no lumina, the cells and nuclei are much larger than in the other glands (mucous average about 11 microns in greatest diameter, poison about 20 microns) and stain differently; and above all the character of the secretion is vastly different.

As might be gathered from the name often applied to them, the secretion in the poison glands is composed of granules. These are of varying size, and the cells are entirely filled with them. The mass stains from red (Pl. XXIII, Fig. 31, *sec.*) or reddish yellow to a dark purple in Mallory; in Van Gieson the

color is as a whole yellow with a tinge of red. In iron haematoxylin some granules stain (Pl. XXII, Fig. 18) black; but at times one can detect in some granules a clear outer portion which takes the counter stain (erythrosin, etc.), while the central part stains dark black, and others which take only the counter stain.

The mucous secretion, on the other hand, reacts very differently, as does the cytoplasm of the mucous cells, which can be easily distinguished from their secretion. Here the reactions are typically those of mucus. Mallory's stain, which colors mucus in the sublingual of a cat a clear blue in two minutes, stains in the same way both the cells and the secretion of the small glands. This same stain beautifully differentiates the mucus in the goblet cells of the oesophagus and intestine of Plethodon. I have not been able to obtain the reaction in these gland cells with thionin, in which Nicoglu places so much confidence as a mucous stain. Hubbard ('03) has had the same difficulty. However, mucicarmine, a specific mucous stain, gives the mucous reaction after twelve or twenty-four hours in both the glands of Plethodon and the sublingual of the cat. The use of Van Gieson's stain clearly differentiates the small gland from the large ones. In the former the cells and the secretion are stained a clear red or pink, without a trace of yellow as in the poison glands. Orcein also, which has been described as a mucous stain, colors the cytoplasm of the mucous gland cells a deep brown, and has absolutely no effect on the granular secretion of the poison glands. The iron haematoxylin is of little use in revealing the mucous nature of the small glands, since they take only the counter stain except for the nuclei. These become a deep black as already said. But this method at least serves to distinguish the two sorts of glands aside from the nuclear staining, in that the secretion of the small glands never takes the haematoxylin, as do the granules of the large glands.

From these distinctions as to the primary character of the two classes of glands, we are led to consider the histogenesis of the secretion. It has been generally held that this process is not the same in the mucous and poison glands. Seeck ('91), p. 55, holds that the secretory cells are of two sorts, "solche die als

Zellen erhalten bleiben und Drüsensecret secerniren (Schleimdrüsen), und andere, deren Protoplasma sich in feinkörniges Drüsensecret metamorphosirt wobei die Zellen vollkommen aufgebracht werden, zu Grunde gehen, so dasz man ihre in Zerfall begriffenen Kernen in Drüsensecret finden kann (Körner-oder Giftdrüsen)." Nicoglu ('93), p. 447, finds that the cells of the poison glands "wenn ihre Stunde gekommen ist, wandeln sie sich in toto in Secretmasse um." But up to this time they act as other gland cells in elaborating and retaining a secretion in their interior, as the pancreas cells do zymogen granules. Schultz ('89) does not think that all the cells of a gland are destroyed at the same time, but such as do form a part of the secretion mass must be regenerated; indicating that they are destroyed in the process of secretion. Drasch ('94) merely states that the poison glands of the salamander, if completely emptied, pass entirely away, and are replaced by new glands. Observations of the glands at various times after emptying show regressive changes in all the layers. Vollmer ('93) also has described the process of solution of the Leydig cells after strong electrical stimulation of the glands, and has made careful statements regarding the appearance of the emptied glands. The conditions in Plethodon almost duplicate those he has described.

It seems pretty well founded, then, that the poison gland cells pass bodily into the secretion mass. But a distinction should be made here, as Nicoglu has done, between the secretion mass as that thrown out, and the secretion material, which is the formed substance in the cells. There is no evidence of the disintegration and solution of cells in the full but not discharged gland. It is only when for some reason the glands are emptied that the degenerative processes are discerned. Otherwise the formed secretion is retained within the cells, which remain in a normal condition at such times.

This review of the literature describes very well the processes which go on in Plethodon; Pl. XXI, Fig. 17, will show the appearance of a gland on the day it was emptied. It has shrunken greatly in size; as compared with others of the same animal which, for some reason were not emptied, from three hundred microns in diameter, say to one hundred microns. The

nuclei which in full glands lie at the bases of the cells, are in this case in the inner parts of the cells, and are larger and clearer and in a state of disintegration. In some places only outlines or shadows of nuclei can be seen. Often they became shrunken and irregular in outline when the gland is emptied. The entire appearance of emptied glands would lead to the conclusion that their time of functional activity is at an end.

The mucous glands, on the other hand, never reveal such changes. It seems correct to say that the processes there are like those in milk glands, where parts of the cell bodies are thrown off as secretion, while the remaining portions in time repeat the same processes of secretion. Nussbaum ('82, p. 302) speaks of the heads or inner portions of the mucous gland cells of Salamander as discharged on stimulation.

If it is true then, as it seems to be, that the poison glands are changed bodily into the mass of secretion, we must look to some source for their replacement, if the animal is to have their continued protection. Nussbaum's conclusions should be cited here ('82, p. 336) as bearing on the general topic of death of gland cells through secretory activity, and their renewal. He says secretion consists in the formation and elaboration of the mother-substance of the secretion material, the changing of this in the cells and in emptying the secretion when ready, out of the cells. "Wie alles Lebende aus uns unbekannten Ursachen abstirbt und neuen Generationen Platz macht, so gehen auch nach einer gewissen Zeit Drüsenzellen zu Grunde und werden von lebenskräftigen Nachbarzellen ersetzt. Sterben aller Zellen gleichzeitig ab, so ist die Drüse vernichtet wie eine Protozoen Colonie. . . . . Die Secretion mag wohl die Zelle abnützen; die Zelle wird altern. Der Ort der Secretion ist aber nicht gleich bedeutend mit Zellentod; er ist eine energische Lebensthätigang."

In this particular case of the skin glands of Amphibia, a definite process of replacement goes on, occurring in Plethodon in the way described for other salamanders by Nicoglu ('93), Heidenhain ('93) and Vollmer ('93), and not as Junius ('98) claims, by entirely new origin. The former observers find that inside the old poison glands there lies a second smaller gland, possessing a lumen. This small sac is to replace the older gland

and lies always between the musculature and epithelium of the latter. Nicoglu ('93) finds that the new glands possess "all the epithelial parts of the old gland with the exception of the "Schaltstück." Whether this statement is to include also the muscle fibres, he does not say; his figures show muscle cells lying upon the ingrowing gland, but there is no reference to prove that they belong to it rather than to the old gland. However, Vollmer ('93) says that the new gland contains both gland cells and smooth muscle fibres, which arise as does the gland bud, from the Malpighian layer of the epidermis.

The place of origin of the replacement glands is found by Nicoglu ('93) and Heidenhain ('93*a*) in the very small, flattened cells immediately adjoining the Schaltstück and lying inside the gland. Vollmer ('93) on the other hand concludes that the place of origin of the new gland "ist das Keimlager des Rete Malpighi. Auch die von Heidenhain erwähnten unscheinbarer Zellenelemente, denen er die Bildung der Drüsenknospe zuschreibt stammen vom Rete Malpighi." There is no reason, he says, why the new glands inside the old ones should not differentiate as do the first glands in the course of their development.

In Plethodon the method of renewal of the worn-out glands is as these authors have described, but there is no evidence showing the source of the replacement glands, and the subject must be dismissed with the above references to the literature.

But whatever the source of the new glands, there can be no doubt that in every old gland without exception there is a small sac or replacement gland. This is always found in those glands which have not been discharged (Pl. XXIII, Fig. 31), as well as in those which have been and show the most extensive degenerative phenomena. In this respect Plethodon seems to differ from Triton (Vollmer '93). This author states that the growth of the new gland is initiated when the old glands are emptied. Nicoglu ('93) mentions the fact that the old poison glands contain the smaller sacs, but does not say definitely whether or not the destructive processes must have set in before the the new gland makes its appearance. But in Plethedon the *presence* of the replacement gland is not dependent on the secretory processes in the large glands. The former are present in the glands of an

animal thirty-eight mm. long which are not filled with secretion.

We have to deal then, in these cases, with the regeneration of a gland by a gland. Individual cells are not broken down, and then renewed by the growth of new cells as Schultz ('89) maintains, and as seems to be implied by Calmels ('83), who finds that the young gland cells are indifferent elements which may develop into either poison or mucous cells, so that a gland may be poisonous only in part.

The question, however, as to whether a poison gland is replaced only by a poison gland is still to be considered. May not these be renewed by glands which to begin with are mucous in character? That is, may not a specific poison secreting epithelium be replaced through mucous cells, and gland by gland instead of cell by cell? These inquiries have been raised by Nicoglu, and he says ('93, p. 425) that a mucous cell never goes over into a poison cell, or vice versa, and Schultz ('89) also says that mucous glands are always only mucous glands, and poison glands only poison glands (p. 33), and therefrom we are to suppose that the same is true of the individual cells, as he finds that cells replace cells.

Still the evidence gained by a study of the poison glands of Plethodon indicates rather strongly that we have to deal with *a production of poison glands from mucous glands entirely.* Nicoglu has already shown that in Triton a mucous gland may sometimes replace a poison gland entirely, but he very strongly opposes the idea that the function of such a gland ever changes. He holds (p. 435) that the condition of mucus within poison gland is a functional adaptation, because the animal needs more mucous glands than are on hand. Everything goes to show that in Plethodon, on the other hand, the occasional method of regeneration described by Nicoglu is the only one. The replacement glands already described stain blue without exception in Mallory, which has been shown to be a mucous stain. The contrast between the blue of the mucus and the red of the granular secretion is very sharp (Pl. XXIII, Fig. 31). The mucous reactions described for Van Gieson, orcein and mucicarmine, are shown invariably in the replacement glands as in the mucous glands outside, and the correspondence of the replacement

glands stained in iron haematoxylin with the other mucous glands is just as complete (compare Pl. XX, Figs. 2 and 3).

There can be nothing clearer than the reaction of the new glands to Mallory's stain. The blue color is present in every case as shown by a study of hundreds of glands. In the very large glands on the back of the tail the ingrowing glands never reach beyond a certain size, such as is shown in Pl. XXIII, Fig. 31. This may possibly be due to some effect of the poison which would hinder the growth of the small gland, or, as seems more likely, the new gland does not develop because it is hemmed in and hindered in its growth by the pressure of the large amount of secretion in the old gland. Drasch ('94) has made this suggestion previously, but does not say where the replacement glands are located. But in all the small poison glands which lie along the sides of the tail and also on the dorsal and ventral surfaces, particularly in the constriction, can be seen all stages of development of the mucous glands within them, from the small buds to new glands which have almost entirely replaced the old ones. The small poison glands differ from the largest ones in no other respect than in size, and for that reason it seems fair to conclude that the processes of regeneration going on in them are characteristic, and typical of those believed to occur under certain circumstances in the large glands of Plethodon, and as observed in other salamanders. There are many cases to be seen in Plethodon in which some glands are so far replaced by a new mucous gland that only a faint crescent of granular secretion can be seen, the rest of the contents being mucus. In other cases the amount of granular material is a little greater, and in still others we may see the gland half granular and half mucus (Pl. XX, Fig. 3; Pl. XXII, Figs. 18, 19, 20). In all these the granular portions stain as do the same parts of the large glands, while the remainder reacts to Mallory and the other stains as do the small sacs in the large glands and the mucous glands outside of these.

To sum up the foregoing we may say that the small glands within the large ones react like known mucous glands to Mallory's stain and mucicarmine, and in the same way so far as the nuclei of the replacement and mucous glands of the tail are concerned,

to iron haematoxylin. That is, both the mucous cells and those of the replacement glands stain blue in Mallory, red or pink in Van Gieson, and both have a fibrillar structure. The mucous reaction is also given with mucicarmine. And finally, the nuclei of the ingrowing gland fundaments always stain intensely black in iron haematoxylin, as do the nuclei of the mucous glands.

The facts just related have been gained entirely from a study of preparations made from material taken from unstimulated animals, that is those not irritated prior to immersion in killing fluids. The evidence along this line is stronger and more convincing in the case of an animal which, without stimulation of any kind other than such as might have occurred in nature, got rid of a great deal of the secretion in the glands of the tail and then cast that organ off, as if it could be of no further use. The animal in question, when first observed, was seen to be entangled head down between some pieces of bark in the terrarium in which it was confined. This seemed to irritate the salamander very much, for when it freed itself it began moving quickly about, swinging its tail from side to side like an angry cat. The tail, during this time, became covered with a very abundant white secretion, After about five minutes of such behavior on the part of the animal, when I merely touched the tail it was suddenly thrown off, the break being in the constriction back of the cloaca.

The tail was put into Zenker's fluid after about fifteen minutes, and sections made later. Here the likeness between the fundaments in the empty poison glands and the mucous glands could not be more complete. In all the stains used the appearances are exactly the same. The cells of the mucous glands are much higher than in other animals seen, stain a lighter blue in Mallory, and have a vesicular structure approaching granular, rather than the filar structure usually seen. Even so, the replacements glands cells are their exact counterparts, and show the same reactions to Van Gieson, mucicarmine, and iron haematoxylin, as well as Mallory's stain.

It seems hardly possible that the cells of the mucous glands could have so changed their structure and appearance in fifteen or twenty minutes, though the increase in height and consequent

diminution in size of the lumen of the gland, together with the vesicular structure of the cells, would lead one to think that they are in the way of becoming granule or poison cells. But whatever the interpretation put upon this appearance, and to whatever source it is due, it must be admitted that the fundaments in the old poison glands have undergone the same processes and their histological characters are now exactly similar to those of the mucous glands.

Further evidence that the glands are originally all of the same character may be gained from the literature. Ancel ('02), who has followed very closely the development of the skin glands in salamander, considers that the large glands represent organs more completely differentiated than the small glands toward a special functional adaptation, though both in early development are absolutely alike (pp. 269, 283.) Junius ('98) believes that there is but one kind of gland in the skin of the frog and probably of all Amphibia, and that the various glands of the authors are young and old forms or developing stages of them. He says further that in the frog he has not seen the regeneration described by Vollmer and Nicoglu, and declares that atrophied glands are replaced by wholly new ones developed by downgrowths of epiderm cells into the cutis. According to him, small glands represent young stages of large ones, and the former are equivalent to the non-contractile or mucous glands, while the latter are the dark, contractile, granule or poison glands.

Again, Hoyer ('90, p. 354) finds that in some poison glands of the salamander single cells or groups of cells lying between the non-staining large granular cells take on a red-violet color in thionin (which he employs as a specific mucous stain). He makes the suggestion merely: "Möglicher Weise deutet dieses eigenthümliche Verhalten auf eine genetische Beziehung der in den Drüsenzellen enthaltenen mucinähnlichen Substanz zu dem giftige Secrete." And finally, the observation of Phisalix-Picot ('00) that the secretion of the mucous glands of the Salamander is poison, seems to me to bear along this line of a correlation between the so-called mucous glands and the poison glands.

Evidence in this direction also, further than that already advanced seems to be indicated in the poison glands of Plethodon.

Here there is very frequently a distinct blue tinge to the *granular* secretion. This may possibly be because the metamorphosis from "a mucus-like substance to the poison secretion" is not entirely completed. At any rate one is impressed with the likelihood that there is mucous material in the poison glands outside of that contained in the replacement glands.

In the discussion of the replacement of the poison glands by those of the mucus variety, it has been shown that every large gland has within it the fundament of a new gland which to all stains for mucus except thionin gives the mucous reaction, and which is also the exact counterpart of the small glands having the mucous secretion. The fact that only in poison glands of smaller size are found evidences that they are entirely replaced by mucous glands, may be explained on the ground that there the amount of granular secretion is not sufficient to mechanically hinder the growth of the new replacement gland. The actual transition stages from mucous to granular secretion have not been observed in my material.

If we make the assumption in view of these facts that the glands of mucous character in the poison glands develop only into mucous glands on the death of the latter, we are forced to one of two conclusions: either that the small glands *outside* the large ones, especially in Plethodon on the dorsal surface of the tail, become the poison glands, or, on the other hand, that when the latter are once destroyed there is no return to such structure except by developing anew according to the embryonic type.

The latter process is going on continually in large as well as in small animals, as can be readily seen by inspection of sections. But it seems that the fundaments are all alike to begin with (Ancel '02); as this author says, the solid gland buds in which a cavity is formed do not undergo further important morphological transformations, and constitute the mucous glands. Those which remain solid, however, continue their development in other ways and form the poison glands (p. 269). It seems to me that this is equivalent to saying that in embryological development the poison glands pass through a mucous stage to reach their final form and character. It certainly lends evidence to the view

expressed, that the glands which are to replace the worn-out poison glands are originally mucus in character.

There is no reason to believe, however, that the replacement glands are functionless during the life of the poison glands in which they lie. Even the smallest replacement glands have distinct ducts and epithelium, and in some cases it is absolutely certain that they have elaborated a secretion similar in every respect to that of the mucous glands.

It is very probable that under all ordinary conditions the small glands in the large ones secrete mucus, and in this sense are adaptations; not because the animal through some unusual external conditions has come to need more mucous glands as Nicoglu ('93) says, but rather because under normal environment there is always need of more mucus than can be secreted by the glands outside the poison glands, especially when the latter are so closely crowded together as on the back of the tail in Plethodon. And much evidence goes to show that under stress of necessity such mucus secreting glands become by replacement the more highly specialized poison glands and take on a particular function, that of forming a substance protecting the animal from its enemies (Hubbard '03.)

The nerve supply of the skin of Amphibia has been a favorite subject of study for many years. Most investigators have limited themselves to the terminations in the sense organs of the skin and in or on the ordinary epidermal cells (Pfitzner '82; Canini '83; Frenkel '86; Massie '94; Herrick and Coghill '98; Coghill '99). The innervation of the glands has received less attention.

Eckhard ('49) first showed that the glands could be emptied by stimulating the anterior roots of the cerebro-spinal nerves, but did not consider the structure of the nerve endings. Eberth ('69) found that there is a network of very fine fibres close upon the glands; Englemann ('72) came to the same conclusion and showed farther that from the nerves about the gland fine twigs are given off to the contractile cells. Openschowski ('82) describes a network of nerves surrounding the glands, as well as an intracellular net; but from his figures it is hard to believe that the structures he shows are nerves. Drasch ('89) also experimentally proved the efficacy of nerve stimulation in obtaining

secretion from the glands, as does Phisalix-Picot ('00). Eberth and Bunge ('92) have described free nerve fibres which seem to end with knobs outside the epithelium of the ball of the thumb of the male frog. Loeb ('96) has also shown how closely the glands of Amblystoma are connected with the central nervous system. In 1898 Herrick and Coghill were able to show the existence an intimate connection of nerve fibres with the walls of the glands, but were unable to discover the exact relation of the fibres to the gland cells. They also described the plexus of nerves beneath the corium as being composed of two sorts of fibres; larger ones connected with the nerve bundles of the central system, and smaller ones which in part, at least, originate in ganglion cells in the corium. Schuberg ('03) has criticised the results of these authors, contending that many or all of the nerve bundles described are really connective tissue bundles, and that the ganglion cells are the "Mastzellen" he himself figures.

Massie ('99) continuing the work of Herrick and Coghill, considers the same arrangement of fibres beneath the corium, and also shows that nerves end on the muscles of the "ental" glands. He finds that nerve fibres passing from the nerve bundle plexus under the corium are intimately connected with the ental glands, and seem distinct from the nerves supplying the muscles. "It seems, therefore, that there are two groups of nerves passing to the glands of the ental series; the one attaching by the typical endings to the enveloping muscle cells, the other ramifying promiscuously over the surface of the gland." (p. 59.)

In the study of the nerves of the poison glands of Plethodon, three methods have been relied upon; namely, the silver nitrate-pyrogallic acid method of Cajal, and Mallory's phosphotungstic acid haematoxylin and fuchsin-orange G-anilin blue methods. The last named gave most excellent results, while of the other two Cajal's was only indifferently successful.

The haematoxylin of Mallory stains only the sheaths of the nerves and so it is of no value in tracing the axis cylinders, since, as is well known, the nerves lose the medullary sheaths on passing into the corium. Beneath the corium, however, the nerves can readily be followed by this method. In some instances fibres

are shown running for long distances beneath the corium, and branches can even be seen to turn toward the epidermis, but all traces of them are lost as soon as they enter the corium.

The other method of Mallory gives like results as far as the distribution of the nerves beneath the corium is concerned. In cross sections of the tail it is often possible to trace a fibre from the roots leaving the cord out to the corium. Sometimes this may be seen in one section; in many cases two or three neighboring serial sections will show the same. The plexus beneath the corium is shown best, as a whole, in frontal sections of the tail. Here it will be seen that the nerves are *very numerous*, and with the method in hand can be traced to their connections with the cord. There can be no question as to the presence of the nerve-bundle layer of the plexus that Herrick and his pupils have shown; but as regards the stratum of glanglion cells, it seems to me that Schuberg's criticism holds good. At any rate neither of Mallory's methods reveals such a structure, and this would at least seem strange in view of the beautiful staining of other nervous elements. In cross sections of the tail, Mallory's fuchsin method shows nerves running in or immediately beneath the inner corium layer. At times several fibres are in view at once, being, however, of different sizes.

Within the corium the distribution of the nerves to the glands is not apparent in sections which pass through the gland, owing to the exceedingly small size of the fibres. But when the periphery of the gland is just denuded, the nervous elements are shown very clearly. In such cases it will be seen that there is a feltwork of many *verg fine fibres* closely investing the gland, *ending upon the muscle fibres* and *around the nuclei of the gland cells*.

The endings upon the muscles are shown both by Cajal's method and Mallory's fuchsin stain, and in some cases are typical (Pl. XXII, Figs. 25 and 26) as described by Huber and Dewitt ('97) and Coghill ('99). That is, they are equipped with terminal expansions or bulbs which lie on the muscles. In many cases fine branching fibres can be clearly seen lying upon the muscle layer. These pass over ultimately into the finest of slender twigs which without terminal expansions always lie on a muscle fibre and end there (Pl. XXII, Fig. 26.)

The fibres in the perinuclear endings are of much the same character as those of the muscles. There are many instances which are very clear of basket structure about the nuclei of the large glands (Pl. XXII, Figs. 21, 22. Pl. XXIII, Fig. 30). I have not been able to discover connections between the fibres and the nuclei, though in at least one case (Pl. XXII, Figs. 23, 24) the fibres end in knobs which lie directly on the nucleus. The latter seems usually to be surrounded only by a basket of fine fibres. Bethe ('94) has described three sorts of endings on cells. Of these he finds that in the unicellular glands of the frog's palate one frequently finds under the nucleus a small blue knob which is connected with a fibre. The latter cannot, however, be followed farther.

In the case of the gland cells under consideration, there can be no doubt that the nuclear basket is connected with nerve fibres. That there should be a nerve supply to the gland cells, seems evident from the experiments of Drasch ('89), Eberth ('49) and Loeb ('96) on Amphibian glands, and we have in Plethodon histological evidence of such supply. The well-known influence of the nervous system on the secretion of sweat, for example, may be also mentioned in this connection. Herrick and Coghill ('98, p. 51) have suggested the possibility of a connection between the nerves enveloping the glands, and the gland cells, but were not able to demonstrate it.

The objection may be raised that we are dealing here with elastic instead of nerve fibres. This does not seem possible for several reasons. The elastic fibres, as has been said, show very little variation in size, and never, as shown by staining in orcein, reach the excessive fineness of the nerve fibres. The branching of the elastic fibres is much less frequent than that of the nerves, and, in clearest distinction the former, as seen upon the glands, take an almost uniform direction even in branching, straight toward the epidermis, while the nerve fibres cross and recross and branch in all directions, and the finest twigs show varicosities which are never seen on the elastic fibres. The general effect of the brown fibres in an orcein stain is entirely different from that of the red ones in Mallory's stain, and leaves no doubt of the distinction here set forth between the elastic and nervous fibres.

SUMMARY.

1. The skin glands of *Plethodon oregonensis*, as of most Amphibia, are of two kinds: granular and mucous. The two are distinguished by the character and staining reaction of their secretions, and by other histological features, as well as by the sizes of the glands.

2. The bodies of the large glands possess an investing musculature, and in addition the ducts have both dilator and constrictor muscles lying in the epidermis.

3. The granule glands are poison in character.

4. In the elaboration and ejaculation of the secretion the poison glands are destroyed.

5. Renewal takes place by the growth into all the old glands of a new and smaller gland, which is mucous in character. The presence of this smaller sac is not dependent upon the removal of the secretion of the large glands, for whether this occurs or not, the fundament giving the mucous reaction is found in all glands; in those which show no degeneration as well as in those where it is wide-spread.

6. The growth of the new gland is dependent upon the removal of the secretion about it. There is evidence that even in case the glands are hindered in their development, they still secrete mucus. But when not hemmed in by the heavy granular contents of the large glands they grow and take the place and very probably assume the function of the old glands which they replace.

7. Both musculature and epithelium of the granule glands have a direct nerve supply. The gland cells are surrounded by a basket work of fibres, which in some cases have terminal expansions lying on the nuclei. The muscles are supplied by nerves with typical endings of expansions or bulbs, as well as by fine twigs without terminal expansions.

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April 29, 1904.

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LIST OF ABBREVIATIONS USED IN THE PLATES.

con.m.—constrictor muscle fibres.
c.t.b.—connective tissue bundles.
c.t.l.—connective tissue layer of gland walls.
c.t.—connective tissue in epidermis.
c.w.—cell walls.
d.—duct of gland.
dil.m.—dilator muscle fibres.
el.f.—elastic fibres.
ep.—epidermis.
ep.m.c.—cell containing musculature of duct.
fl.c.—funnel cell.
i.c.l.—inner layer of corium.
l.d.—lumen of duct.
l.gl.—lumen of gland.
m.b.—muscle bundles.
m.c.l.—middle layer of the corium.
m.f.—muscle fibres.
m.gl.—mucous gland.
m.n.—muscle nucleus.
n.c.—nerve cord.
n.e.—nerve endings.
n.fl.c.—nucleus of funnel cell.
n.f.—nerve fibre.
nuc.m.c.—nucleus of mucous cell.
nuc.p.c.—nucleus of poison cell.
nuc.ep.m.c.—nucleus of muscle cell in epidermis.
o.c.l.—outer layer of corium.
p.fl.c.—processes of funnel cells.
p.gl.—poison glands.
pig.—pigment.
prol.m.f.—prolongations of muscles into epidermis.
rep.c.—replacement cell (and nucleus).
rep.gl.—replacement glands.
sec.—secretion.

All the figures were drawn with the Abbé camera lucida.

ERRATA

P. 251, l. 31: *For* fuchsin-orange G-anilin blue,
read fuchsin-orangeG- anilin blue.

P. 259, under Zalesky 1866: *For* Bd. J 1, *read* Bd. 1; *for* Saeyler, *read* Seyler.

P. 264, under description of Fig. 16: *For* $\times$ 1650, *read* $\times$ 825.

P. 266, under description of Figs. 21, etc.: *For* $\times$ 1850, *read* $\times$ 925.

PLATE XX.

Fig. 1.—Cross section of entire tail, showing position on dorsum of large poison glands (*p.gl.*) and the mucous glands (*m.gl.*) chiefly on the ventral side. Diagrammatic except in outlines and proportions of parts. Van Gieson. × 22

Fig. 2.—Mucus gland from ventral side of tail, showing large lumen (*l.gl.*), and dark staining, angular nuclei (*nuc.m.c.*). Lower part of funnel cell (*fl.c.*) shown in epidermis (*ep*) which is not reproduced entire. Benda's iron haematoxylin. × 342

Fig. 3.—Poison gland (*p.gl.*) of small size partly replaced by ingrowing mucous gland (*m.gl.*). Funnel cell (*fl.c.*) shown in epidermis (*ep.*); nuclei (*nuc.m.c.*) of mucous gland darkly stained as in Fig. 2. Benda's iron haematoxylin. × 342

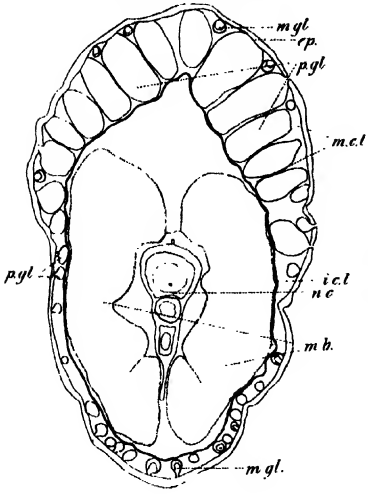
Fig. 4.—Outline drawing of cross section of duct of poison gland showing replacement cells of the funnel (*rep.c.*) rolled one within the other, the funnel cell (*fl.c.*) and the lumen of the duct (*l.d.*) Mallory's conn. tissue stain. × 875

Fig. 5.—Portion of lower part of poison gland showing bundles of connective tissue (*c.t.b.*) passing from the inner layer of the corium (*i.c.l.*) to the connective tissue layer of the wall of the gland (*c.t.l.*) Nuclei (*nuc.p.c.*) and walls (*c.w.*) of gland cells. Secretion not shown in detail. Mallory's conn. tissue stain. × 342

Fig. 6.—One side of median longitudinal section of duct of poison gland showing muscle fibre (*m.f.*) and its nucleus (*m.n.*) and the prolongation of the fibre (*prol.m.f.*) into the epidermis (*ep.*). Compare with Pl. IV, Fig. 27. Mallory's conn. tissue stain. × 280

Fig. 7.—Branching muscle fibres (*m.f.*) from lower part of gland. Mallory's conn. tiss. stain. × 342

Fig. 8.—Longitudinal section of poison gland through the mouth showing two expansions of muscles (*m.f.*) in which the nuclei lie, and portions of muscle fibres. Nucleus of funnel cell (*n.fl.c.*) at duct (*d.*). Secretion of gland not shown. Ferric-chloride haematoxylin. × 342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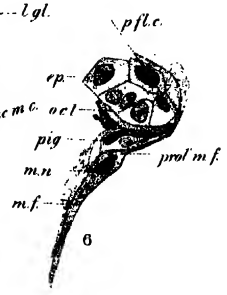
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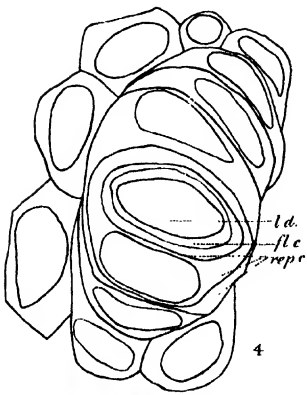
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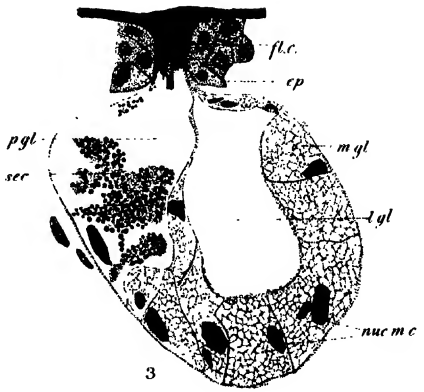
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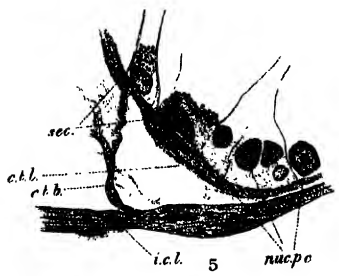
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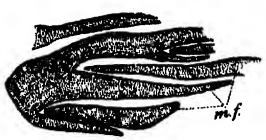
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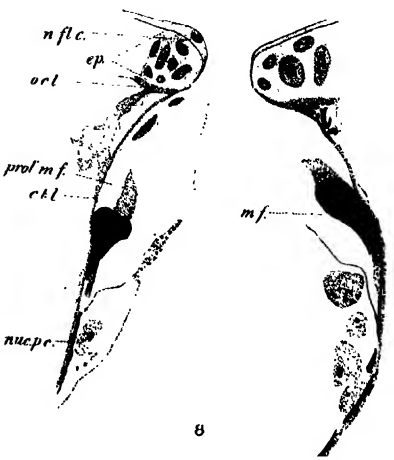
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PLATE XXI.

Fig. 9.—Elastic fibres (*el.f.*) on surface of gland. Gland wall (*c.t.l.*) in section indicated; also nuclei of gland cells. The elastic fibres pass through the inner layer of the corium (*i.c.l.*). Tänzer's orcein. × 342

Fig. 10.—Section through upper pole of gland at one side of the duct, showing cut ends of muscle fibres (*m.f.*) and their nuclei (*m.n.*). From cross section of tail. The nuclei in this figure correspond in position to that shown in Pl. XX, Fig. 6, and to the enlargement of the fibres shown in Fig. 8. Mallory's conn. tissue stain. × 342

Fig. 11.—Tangential section through wall of gland and the mouth, from frontal section of tail. Muscle fibres (*m.f.*) and nuclei (*m.n.*) shown. Funnel cell (*fl.c.*) lining duct and some secretion (*sec.*) in lumen of duct (*l.d.*). Mallory's conn. tissue stain. × 342.

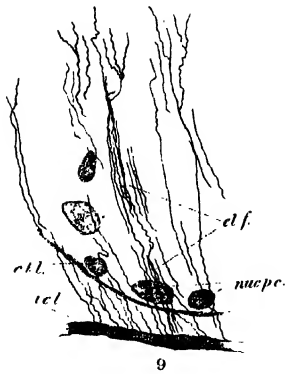
Fig. 12.—Cross section of gland from frontal section of tail, at level of muscle nuclei (*m.n.*). Compare with Figs. 10 and 11. Van Gieron's stain. × 400

Fig. 13.—Cross section of epidermis at upper pole of poison gland, showing deep lying epidermal cell (*ep.m.c.*) which contains the constrictor and dilator muscles of the duct. Mallory's conn. tissue × 342

Figs. 14 and 15.—Cross sections of ducts at level of cell described in Fig. 13, showing constrictor (*con.m.*) and dilator muscles (*dil.m.*). In Fig. 14, only the outer ends of the constrictor fibre appear. In both figures are shown the ends of the muscle fibres (*m.f.*) of the glands, in the epidermis, and the connective tissue (*c.t.*) outside the nuscles. The nucleus of the epidermal muscle cell is shown in Fig. 14. Mallory's conn. tissue stain. × 875

Fig. 16.—Description as for Figs. 14 and 15. But one set of constrictor fibres shown; lumen of duct (*l.d.*) nearly closed. Mallory's conn. tissue stain. × 1650

Fig. 17.—Longitudinal section of nearly empty poison gland. Secretion (*sec.*) very small in amount, cell walls (*c.w.*) distinct, nuclei clear and of irregular shapes. Semi-diagrammatic in unimportant details. Benda's iron haematoxylin. × 342



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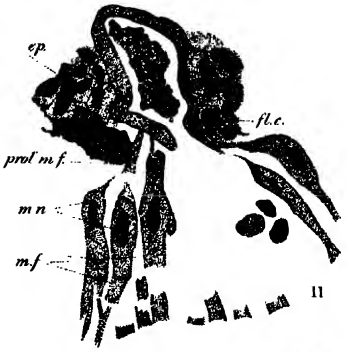

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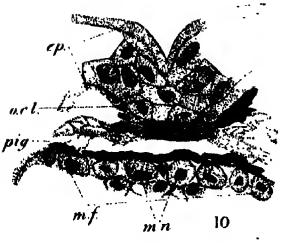

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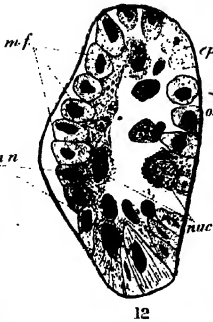

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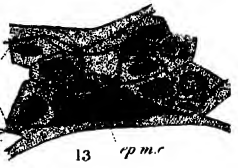

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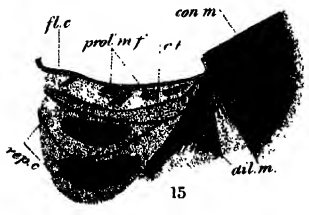

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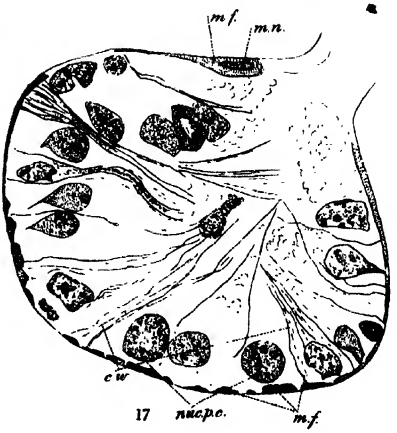

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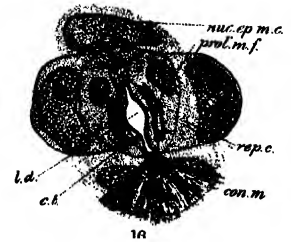

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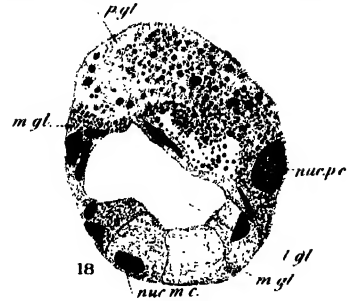
PLATE XXII.

Figs. 18, 19, 20.—Stages in replacement of small poison gland (*p.gl.*) by mucous glands (*m.gl.*) from sides of tail. Mucous nuclei dark. Secretion (*sec.*) shown in poison part only. Benda's iron-haematoxylin. × 342

Figs. 21, 22, 23, 24.—Tangential sections of poison glands, showing nerve endings (*n.e.*) on nuclei of poison cells (*nuc.p.c.*). Mallory's conn. tissue stain. Figs. 21, 22, 24. × 1850. Fig. 23. × 875

Figs. 25 and 26.—Tangential section of wall of poison glands, showing nerve endings (*n.e.*) on muscles (*m.f.*). Fig. 25, Mallory's conn. tissue stain. Fig. 26, Cajal's silver nitrate-pyrogallic acid. × 875



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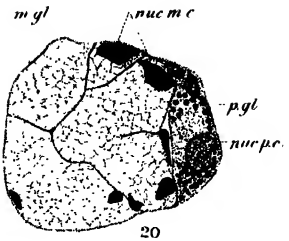

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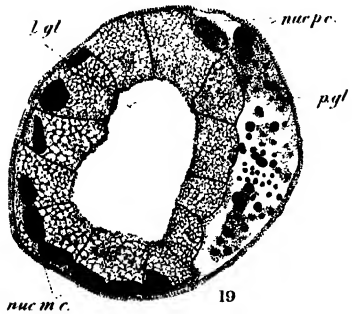

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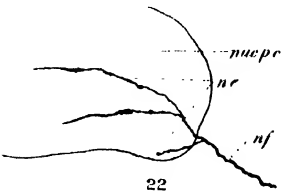

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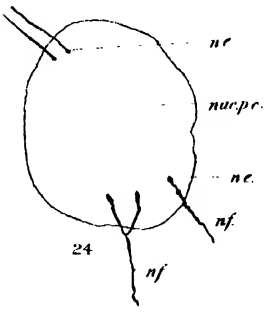

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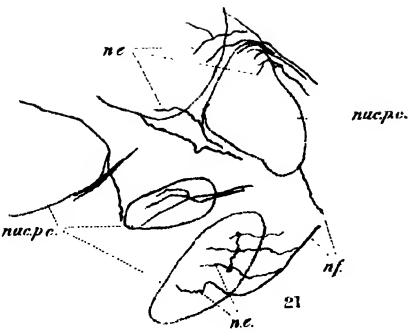

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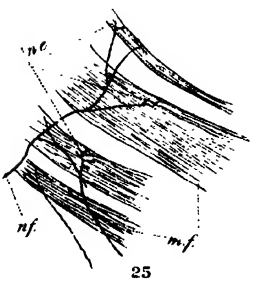

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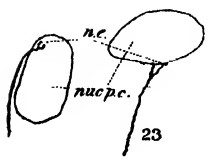

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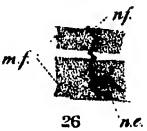

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C. O. E. DEL.

PHOTO-LITH. BRITTON & REY. S.F.

PLATE XXIII.

Fig. 27.—Median longitudinal section of duct of poison gland, showing prolongation of funnel cell (*p.fl.c.*), prolongation of muscles (*prol.m.f.*) into the epidermis, and the connective tissue (*c.t.*) outside them. Replacement cells (*rep.c.*) shown with processes extending down as far as funnel cell. Mallory's conn. tissue stain. × 1650

Figs. 28, 29, 30.—Cross sections of ducts, showing funnel cells (*fl.c.*), gland muscles (*prol.m.f.*), connective tissue (*c.t.*) at sides of duct (*d.*), and constrictor and dilator muscles (*con.m, dil.m.*). Mallory's conn. tissue stain. × 1650

Fig. 31.—Longitudinal section of poison gland, showing small mucus gland (*m.gl.*) inside it. Large gland 440 microns by 180 microns; small gland 90 microns by 43 microns. Mallory's conn. tissue stain. × 1650

Fig. 32.—Nerve-endings (*n.e.*) about nucleus of poison cell (*nuc.p.c.*). Mallory's conn. tissue stain. × 1650

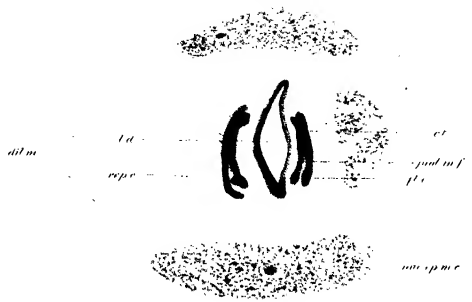


Fig. 28

Fig. 27

Fig. 29

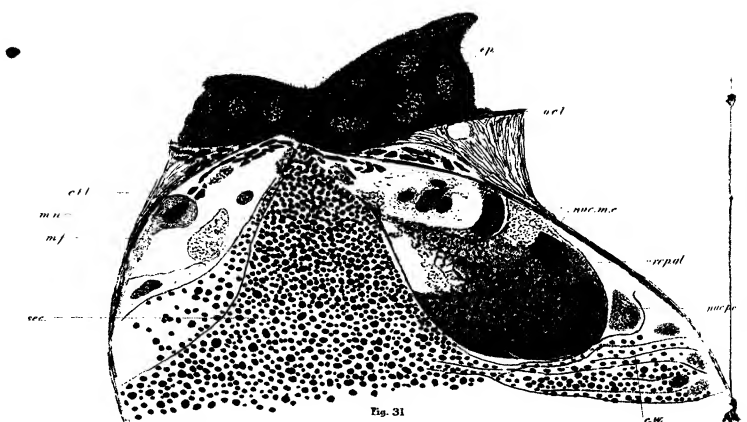


Fig. 31

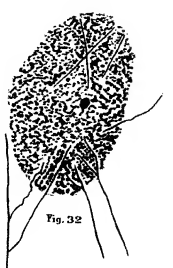


Fig. 32

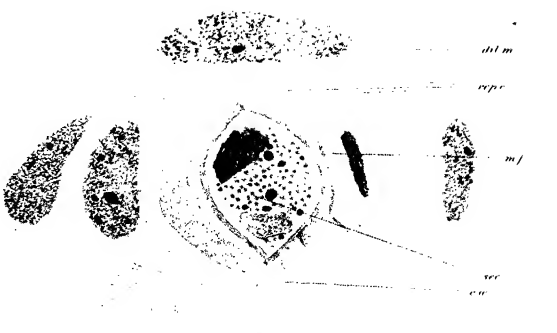


Fig. 30

It is the purpose of the present paper to describe the distribution of the sense organs over the body of *Microscolex elegans*, a California earthworm, and to make some comparisons with *Lumbricus agricola* as described by Miss Langdon. These two worms are quite different as regards structure and habits. No effort was made to work out the connection of the sense organs to the nervous system of *Microscolex*.

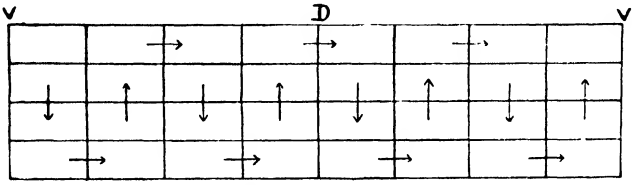
MATERIAL AND METHODS.

Microscolex is a small worm with apparently a limited distribution. It has been found in Berkeley, but is far more abundant in Golden Gate Park, San Francisco. Dr. Eisen ('94) has reported it also from Mount Diablo and Sonoma County regions. The worm is usually found in manure piles or amongst decaying leaves. In Berkeley a pile of leaves was found which contained no other forms than *Microscolex elegans*, while in the park in San Francisco it was associated with *Allolobophora foetida*. It was noted that *Allobophora calignosa*, a worm very common throughout the State, was seldom found in the immediate vicinity with these worms.

Fresh material was used wherever possible, but after the dry weather came preserved material had to be resorted to. The worms were allowed to swim about a while in water to free them from grit and dirt, and were then killed by allowing alcohol to drip into the dish at the rate of 60 drops to the minute. In this way the worms were stupefied in from two to three hours. From the drip they were transferred to 80 per cent. alcohol and held between glass rods to keep them straight while hardening. When hardened they were preserved in 95 per cent. alcohol. Material to be used in sectioning was taken directly from the drip and killed in Flemming's solution or corrosive sublimate and acetic acid. Sections 2 to 5 microns in thickness were stained with Mayer's haemalum or Delafield's haematoxylin. On the whole, material stained in toto with Delafield's hematoxylin gave the most satisfactory results.

The cuticle was prepared in the following manner. Freshly killed worms were split open along the mid-ventral line from the mouth to the anus. They were then put into 30 per cent. alcohol or water to macerate, when after two or three hours the

cuticle could be removed. They are very delicate and easily torn, so as to insure removal without tearing the worm was cut transversely into two parts and the cuticles handled with camel's hair brushes. This is practically the same method used by Miss Langdon ('95). They were then carefuliy spread out on slides and allowed to dry. When thoroughly dry the cuticle sticks to the slide and is ready to be examined. The spots which indicate the areas of the sense organs are thinner than the rest of the cuticle, and perforated with minute holes where the sense hairs project. At best these areas are not clearly defined. Staining the cuticle was tried with very good results, this bringing into stronger contrast the sense areas and the remainder of the cuticle. The staining was done before the cuticle was spread on the slide. The cuticle was dipped into a strong stain two or three times, rinsed with pure water and then mounted. A few cuticles were stained after mounting, but on account of their hardness when dry, they did not take stains well. Nigrosin, iron haematoxylin, Ehrlich's haematoxylin and methylin blue were used. Nigrosin was the most satisfactory. The staining of the cuticle is an important aid to accurate study of the distribution of the sense organs.



Text Fig. 1. The small rectangles indicate the areas as seen with the small diaphragm in the eyepiece of the microscope. The arrows indicate the order in which the areas were examined.

The plan was to count all the sense organs found in the entire cuticle and to plot these so that their distribution in any part might be seen at a glance. It was also desired that the count of the sense organs should be as accurate as possible. In order to see the sense areas well a high magnification was necessary. Under these conditions a whole metamere could not be in the field at once, so a method was devised whereby a small area

could be counted at a time. A rectangular diaphram of such dimensions that its width was one-fourth the width of the metamere and its length one-eighth the length of the metamere, was put in the eyepiece. By means of a mechanical stage, the whole area of each metamere was gone over, and plots were made of all sense organs in each of these small divisions. The entire surface of one worm was gone over in this way, and parts of four others were enumerated for comparison.

The work, for the most part, was carried on in the zoölogical laboratory of the University of California, but was completed in the laboratory of the University of Oregon. The problem was suggested by, and the work carried on under the guidance of, Professor C. A. Kofoid of the University of California, and I wish here to express my sincerest appreciation for his kind advice and for his help in the final arrangement of the paper.

STRUCTURE OF THE SENSE ORGANS.

The structure of the sense organs of *Lumbricus* and *Microscolex* is practically the same. In *Microscolex* each organ (Plate XXIV, Fig. 4) is made up of a group of sense cells lying loosely in a cavity surrounded by a layer of boundary cells which form a continuous layer usually one cell thick, but it may be thicker. They are the same as the supporting cells of the epidermis, with the exception that they are usually greatly flattened. The boundary cells are somewhat longer than the epidermal cells, because they are bowed outward and stretch from the elevated area just under the cuticle to the thinned part of the basement membrane beneath. The sense organs are ovoid, the greatest transverse diameter being a little closer to the proximal than to the distal end. The smaller distal end forms a raised spot on the cuticle, which is here thinned and has canals for the passage of the sense hairs. The basal end is usually somewhat flattened, and rests on the basement membrane of the epidermis.

The size of the organs is about the same for both worms. They measure from 80 to 100 microns in height, 18 to 20 in diameter at the top and 40 to 60 in the widest part. In *Microscolex* there is a wider range of variation in the diameter of the top as indicated by the dimensions of the sense areas in the

cuticle. These areas above the organs measure from 6 to 35 microns in diameter. Their average size, however, is about 15 microns.

Lying within the membrane formed by the boundary cells, are the sense cells proper. These are greatly elongated and narrower at the ends than in the middle. The widest part is just below the middle, which contains the nucleus. Their distal ends, which are broader than the proximal, carry stiff bristles, which extend through a short canal in the cuticle. The basal end of the cell is very narrow, and runs out into one or two long processes. Among the basal ends of these cells may be found low cuboidal and sharply pointed columnar cells, which rest on the basement membrane and project upward into the cavity of the organ. Several of these have been observed to have long processes running up along the sense cells, thereby suggesting transition stages in their development. It is often difficult to find the cell boundaries near the top of the sense organ, but in the lower end the cells are quite far apart and sharply defined. The internal structure of the sense organ of *Lumbricus* differs from that of *Microscolex* in that in the former species the cells have the same diameter at the distal and the proximal ends, and stand entirely apart, not showing the close approximation at the distal ends. Miss Langdon found no basal cells with processes projecting up into the cavity of the sense organ, while in *Microscolex* these were plainly seen.

The cuticle has three kinds of openings. We note first the largest openings, such as the nephropores (Plate XXV, Fig. 6), the sexual openings, and the chaetae sleeves (Plate XXV, Fig. 5). When the cuticle is stripped off the cuticular sheathes of the chaetea are removed with it, and in the preparations these lie bent over and close to the cuticle, so that the position of each chaeta is definitely marked on the metamere. The second class of openings includes the sense areas (Plate XXV), which mark the positions of the sense organs. These areas are circular, or slightly oval, and thinner than the rest of the cuticle, and each containing near its center openings for the sense hairs, which became more scattered toward the periphery. The third kind of opening is that of the gland cells of the epidermis. These are

the very small and numerous openings found all over the cuticle. They are about the same size as the openings for the sense hairs. The cuticle is made up of two layers, an outer dense and an inner loose, fibrous layer. The thinning of the cuticle for the sense organ is at the expense of the inner layer. The outer layer contains two sets of fiber, which cross each other at an angle of about 60 degrees, each fiber running spirally about the worm. The openings for the gland cells and the sense hairs are made by pushing the fibers to one side, while for the larger openings there is a distinct rupture of the fibers.

DISTRIBUTION OF THE SENSE ORGANS.

The sense organs were found in all parts of the surface of the body of *Microscolex,* while in *Lumbricus* none were found in the clitellum. The organs were not distributed equally, certain regions having more than others. The largest numbers are present in the anterior segments. Passing caudad they gradually decrease till in the middle region of the worm (segments 18 to 90) they reach a degree of constancy for each successive segment approximating 220. Continuing caudad beyond segment 90, the numbers again increase, but do not become so abundant as in the anterior segments. Thus in one worm the second segment had 511 sense organs, the thirty-fifth had 218, while the ninety-third had 310. The ninety-third was next to the last segment in this worm. The lowest number in any segment was 136, in the fiftieth; the highest was 569, in the fifth. In *Lumbricus* the anterior metameres have the largest numbers, but from here on to the caudal end there is a gradual and uninterrupted diminution in numbers. The total sense organs in corresponding metameres are unequal. *Microscolex elegans* contains on an average 103 segments, while *Lumbricus* has over 150. The following table shows some of the figures for corresponding segments:

	Prostomium and 1st Seg.	10th Seg.	56th Seg.
Lumbricus agricola.....	1900	1200	799
Microscolex elegans.....	536	324	218[1]

[1] This is the number of sense organs in the thirty-fifth segment, which corresponds with the fifty-sixth of *Lumbricus,* whose length is twice that of *Microscolex.*

The total number of sense organs in a single *Lumbricus* was approximated by Miss Langdon ('95) at 150,000, while for a *Microscolex* of 103 segments only 14,787 were found. Thus *Microscolex,* with approximately one-fourth the surface of *Lumbricus,* has only one-tenth as many sense organs.

The largest sense organs in *Microscolex* (35 microns in diameter) are on the prostomium, and the smallest (6 microns) on the clitellum. For the rest of the worm, the organs have about the same average size (15 to 19 microns). Those of the posterior end do not increase in size as do the numbers. In *Lumbricus,* on the other hand, the largest are on the prostomium and the first metamere, and caudad they gradually diminish in size, the smallest being on the caudal segments.

Besides these differences in distribution over the general surface of the worm, there are certain zones in each metamere where there is a perceptible grouping of the sense organs. This distribution differs both antero-posteriorly and dorse-ventrally. In the antero-posterior direction three zones are to be seen. The first, the cephalic or anterior zone, extends from the septal furrow in front of the median zone of the metamere. The surface of the anterior zone is an arc, the convexity of whose surface points in the anterior direction. The nephropores occur in this zone close to the septal furrow. The largest numbers of sense organs are in this zone, and passing caudad they decrease in size and numbers in the successive metameres. For the most part the organs of this zone are most numerous near the septal furrow. They do not occur in a distinct line along the anterior margin, as in *Lumbricus,* but are scattered over a small area or belt. The numbers become fewer as we approach the succeeding zone, making a gradual transition from a region of large to one of comparatively small numbers.

The second zone is a narrow one extending through the middle of the metamere, and consisting of a single row of the largest sense organs found in line with the chaetae sleeves. This is true in all the metameres except the first, where the largest organs are in the anterior zone. It is impossible to make out any distinct groupings about the chaetae sleeves, as was shown in the case of *Lumbricus* by Miss Langdon ('95).

The third zone is the caudal, or the posterior. It occupies an area between the median and the septal furrow, and covers an arc the convexity of whose surface points posteriorly. The distribution here is exactly the reverse of that in the anterior zone. Excepting in the prostomium and the first metamere the posterior zone has the fewest and smallest sense organs. In this zone the organs decrease in number caudad till we reach the sixtieth metamere, where they begin to increase in both size and number, though they continue to decrease caudad in the other zones, resulting in the approximation to the constant number previously noted. The anterior and the posterior margins of this zone have the organs scattered, the majority being in the central part.

In all the segments except the first five and the last five the average diameter of the sense organs in the anterior zone is 16 microns, in the median they are 19 to 22 microns, and but 10 in the posterior. Again turning to *Lumbricus,* we find that, beginning in the second, third and the fourth metameres, a median line of sense organs is prominent, and diminishes caudad. No distinct median zone is here recognized, the median line of organs in line with the chaetae are not separated from the posterior zone as they are in the anterior, so that only two zones are distinguishable, a cephalic and a caudal. Around each opening of the dorsal pores, nephropores and sexual ducts of *Lumbricus* groups of organs were found guarding these entrances. No such distribution was found in *Microscolex,* each opening, on the contrary, being surrounded by a small clear area containing no sense organs at all (Plate XXV). In both the worms the surface of the prostomium and the first metamere are covered with sense organs, so that no distinct zones can be made out. In *Microscolex* the posterior margin of the first metamere contains many smaller sense organs, while the rest of the surface of the prostomium is covered by very large organs. The following table shows the distribution in the antero-posterior direction in the metameres of *Microscolex.*

Segment.	Number of Sense Organs in Zones. Anterior.	Median.	Posterior.
6	293	135	101
8	228	83	70
10	180	81	63
12	148	73	52
[1]14	101	69	34
20	102	64	57
30	92	58	65
40	116	57	74
50	53	32	58
60	78	72	100
90	82	76	107
91	67	61	105
[2]93	88	88	134

The dorso-ventral distribution was not mentioned in *Lumbricus,* but is a striking feature of *Misroscolex,* where four regions—a dorsal, two lateral, and a ventral—may be distinguished. The dorsal surface always has fewer organs than the ventral, while the lateral surfaces always have more than either the dorsal or the ventral. For example, metameres 10 to 20 contain on the dorsal surface a total of 483 sense organs, on the ventral 632, and on one of the lateral zones 724. Recording these as they occur in different metameres of a *Microscolex,* we have the following table:

Number of Segment.	Number of Sense Organs on Surface. Dorsal	Lateral.	Ventral.
10	72	76	60
12	47	80	75
13	44	60	58
15	48	63	55
17	19	63	45
20	29	71	60
35	27	67	36
50	18	50	31
80	30	61	55
93	39	89	78

The only exception to this type of dorso-ventral distribution is in the prostomium and the last segments, and here the organs are more evenly scattered over the surface. The ratio of the number of organs in the ventral zone to that of the dorsal varies

[1] Clitellum.

[2] Next to last segment.

from 6 : 5 to 5 : 3. The greatest contrast is in the middle of the worm, where the ventral organs out-number the dorsal 5 to 3.

The distribution of the sense organs in *Microscolex* is significant when viewed in the light of the habits of this worm. *Microscolex* is a small, frail worm, and its movements are quick and rapid. Backward movement is almost as free as movement in the other direction. Many times during experiments on these worms they have covered a distance of more than half a meter in a continuous movement. While the common *Allolobophora caliginosa* has the ability to move backwards, it never shows this in so marked a degree as does *Microscolex elegans*. I have seen these worms moving backwards freely, not only in the burrows, but also on free surfaces, when no experiments were being tried and there was apparently no occasion for such action. Naturally, we should expect the anterior end of the worm to be the most sensitive, and therefore have more sense organs than do the other parts of the worm. There being in *Microscolex* this backward movement, the posterior end should also be particularly sensitive. Actual count of the organs of these regions shows an increase in the number of organs posteriorly and on the posterior arc of the rear segments. The anterior segments have the largest numbers, which decrease till a region of constancy occurs behind the clitellum; then follows an increase in the numbers from the nintieth segment on to the end of the worm.

Another noticeable habit of this worm is its marked squirming movement, especially noticeable on a smooth surface. There are two reasons why the lateral zones should have more sense organs than the dorsal or the ventral zones. First, along the sides of the worm the nephropores occur. If these pores are places of great sensitiveness, then we should expect to find, as in *Lumbricus,* each nephropore provided with a distinct grouping of the sense organs. As Plate XXV, Fig. 6, plainly shows, there is no grouping at all about these openings. On the contrary, the area just about the pore is usually quite free from organs. There are no other openings on the lateral surfaces of the body. The lateral sense organs are scattered in *Microscolex,* and the whole surface must do the duty that a group of the

organs immediately about the opening does in *Lumbricus*. Secondly, in the burrow or on a rough surface, the side to side motion, so characteristic of the worms, would make the sides also a region of first contact with the environment, consequently we might expect the body to be sensitive here and to have more sense organs. The ventral surface is seldom off of the substratum, first, because a better hold is gained here with the chaetae; and second, because gravity tends to keep that side in contact with the earth. For these reasons the ventral surface would have more use for sense organs than the dorsal, whose surface would be stimulated only by occasional rather than by constant contact.

The distribution of the sense organs suggests that the surface of the worm is not equally sensitive in all parts. Experiments to determine the sensitiveness were made, with alcohol and acids as irritants, sugar and quinine as taste stimulants. A fine capillary pipette was used for applying alcoholic solutions of 1 per cent. and less, so that a small quantity could be applied to a small area. The time between the application and some direct manifestation of irritation was recorded by a stop watch. The records show that the anterior end is more sensitive than the posterior, and the posterior more than the middle part. Solutions of quinine of one thousandth of 1 per cent. and even less gave the same results as the alcohol. The fact that the animals reacted to the quinine may indicate that the sense organs have some gustatory function. This might be expected, for the difference in structure between sense organs on the outside of the body and those of the pharynx is very slight. The sense organs of the pharynx are lower and a great deal broader than the others. The results of the last set of experiments are of importance because they show that the degree of sensitiveness inferred from the differences in time reactions of a given region is correlated with the number of sense organs found therein. The ratio of the number of organs in the anterior end to those of the posterior is 3 : 2, while the ratio of time reactions is approximately 4 : 7. Thus the ratio of the numbers of sense organs in two given areas is approximately inversely proportional to that of the time reactions. This relation is shown in

the comparison of the anterior and the middle parts of the worm. The ratio of the sense organs in the two regions is 3 : 1, while the ratio of the time reactions is 4 : 9. In the following table some of the time reactions are given, the time being given in seconds:

Stimulant.	Time Reactions of Different Portions of the Worm.		
	Anterior.	Middle.	Posterior.
Alcohol	2	7.8	5.6
Alcohol	3.8	10.2	7
Alcohol	1.8	10.8	8.6
Alcohol	4.8	8	8.6
Alcohol	5	6	4
Quinine	4.8	5.6	5.6
Quinine	4.6	7.8	6.2
Quinine	7.6	8.6	13.6
Quinine	6	13.6	9

SUMMARY.

1. The anterior metameres contain the greatest numbers of, and the largest sense organs.

2. In the middle of the worm (metameres 18 to 90) a region of constancy is found where each metamere contains about 220 sense organs.

3. Toward the posterior end the number of sense organs per metamere increases to about two-thirds that found in the anterior end.

4. Every metamere shows an antero-posterior and a dorso-ventral distribution of the sense organs.

(*a*) In the antero-posterior direction there are three zones—anterior, median and posterior.

(*b*) In the dorso-ventral direction there are four areas—two laterals, a dorsal and a ventral. The number of sense organs in the ventral area exceeds that in the dorsal, and that in the lateral surpasses the ventral.

5. In the posterior end of the worm the antero-posterior distribution is just reversed, the larger number of organs being in the posterior arc of the metamere. This distribution is correlated with the habits of the worm.

6. The largest sense organs are in the prostomium, and the smallest in the clitellum.

7. In each metamere except those of the posterior end the largest sense organs are found in the median zone, the next in the anterior zone, and the smallest in the posterior zone.

8. The total number of sense organs in *Microscolex elegans* is less than 15,000, to 150,000 in *Lumbricus agricola.*

9. There are no groups of sense organs about the nephropores, sexual ducts, or the chaetae sleeves.

10. The sense organs are the most numerous and the largest on those parts of the worm which most frequently come in contact with surrounding objects.

11. The ratio of the numbers of sense organs in two given areas is approximately inversely proportional to that of the time reactions.

University of Oregon,
Eugene, Oregon, May 14, 1904.

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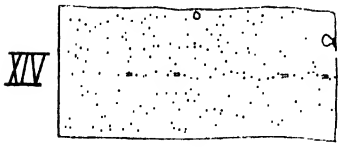
EXPLANATION OF PLATE XXIV.

Fig. 1.—Prostomium and six metameres between the mid-dorsal and mid-ventral lines. The chaetae are represented in the median line in the median zone. The nephropores are seen near the septal furrow. The size of the black dots represents the size of the sense organs only approximately.

Fig. 2.—A metamere from the clitellum. Near the mid-ventral line is the oviducal pore.

Fig. 3.—The next to last and the last metameres of the body, showing the reversed antero-posterior distribution.

Fig. 4.—Sense organ cut in longitudnal section. *cut.*, cuticle; *gl. c.*, gland cell; *b. m.*, basement membrane; *cir. m.*, circular muscle; *b. c.*, basal cell; *s. c.*, sense cell showing one of a group of sense cells and the tapering basal ends; *bd. c.*, supporting cell next to the sense organs; *epi. c.*, epidermal cell; *s. h.*, sense hair protruding through the thinned cuticle.



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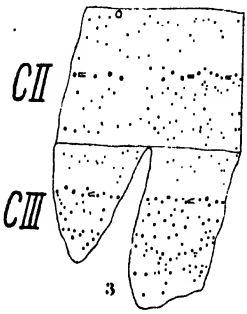

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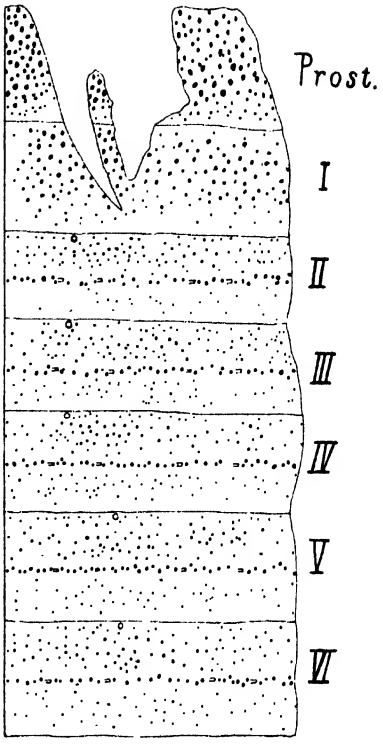

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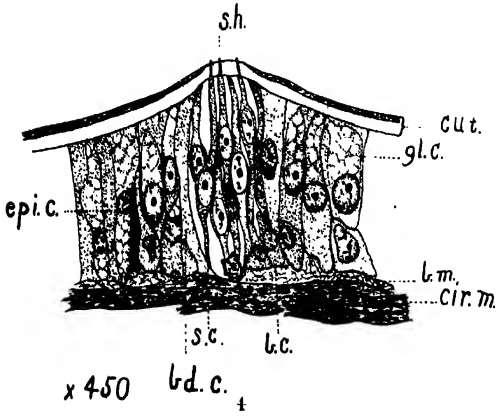

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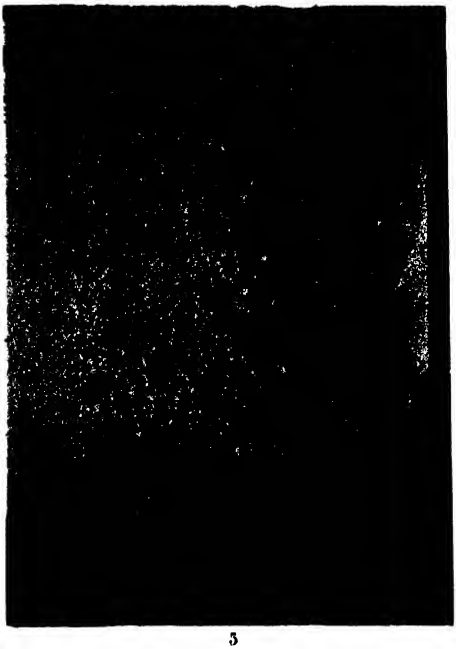
EXPLANATION OF PLATE XXV.

Fig. 5.—Chaetae sleeve, showing the absence of any special distribution about it. The glandular openings appear as black dots in regular rows.

Fig. 6.—Area about the nephropore.

Fig. 7.—The posterior portion of the prostomium, showing the size of the organs in that part.

These micro photographs were taken with a Zeiss microscope, AA objective, and the No. 12 compensating ocular. The magnification in each case is about 88 diameters.



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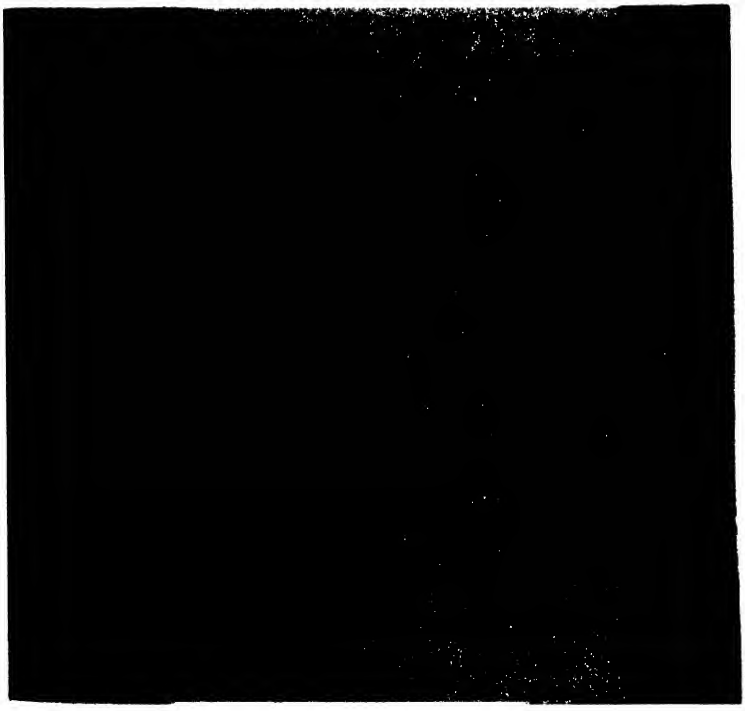

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SOME NEW TINTINNIDAE FROM THE PLANKTON OF THE SAN DIEGO REGION

(From the San Diego Marine Biological Laboratory of the University of California.)

BY

CHARLES ATWOOD KOFOID

The following Ciliates belonging to the family *Tintinnidae* have occurred in the collections made at the San Diego Marine Biological Laboratory in 1903-1905. They appear to be as yet undescribed and are of considerable interest in several instances owing to the highly specialized nature of the external shells or loricae which these simple unicellular animals have formed in adaptation to a pelagic life.

I am indebted to Mr. R. D. Williams and Mr. John F. Bovard, assistants at the San Diego Laboratory, for some of the observations recorded and several of the sketches utilized in this paper.

Tintinnus serratus sp. nov.

Pl. XXVI. Fig. 1.

The lorica of this species is tubular, with slightly flaring ends. Its length is about twelve times its least diameter which is near the aboral end. It gradually enlarges anteriorly, attaining just behind the anterior flare a diameter one and one half times that in front of the posterior flare. Both ends are open, the diameter of the aboral aperture being three-fifths of the oral. Within a short distance of each the wall of the lorica flares

gradually in a regular curve, approximately 30° from the axis, increasing the diameter about 20%. The aboral margin is perfectly smooth but the oral is deeply and regularly incised, forming a serrate margin of twenty erect, acute teeth.

The wall is unusually thin and hyaline even for this thin-walled genus and shows only the faintest traces of structure.

The animal has not been found in the lorica.

The number of adoral ciliary plates in the genus Tintinnus is stated by Daday ('87) to be 18-20. There are 20 circumoral teeth in the lorica of this species, a fact which indicates that there is some correlation between the structure of the adoral apparatus and the formation of the serrate oral margin of the lorica.

This species belongs to the form-cycle of *T. fraknoi* Daday, differing from it in the possession of the serrated circumoral margin of the lorica, and in attaining less than one half its size. As figured by Daday ('87) the ends in *T. fraknoi* flare more gradually and are less differentiated than in *T. serratus*. In the Pacific plankton, however, I find that *T. fraknoi* generally has the flare better developed than it is in Daday's figures of the species from the Mediterranean.

Dimensions:—Length, 150 μ; diameter inside of flare, anteriorly 18 μ, posteriorly 12 μ; of oral opening, 25 μ; of aboral, 15 μ; length of teeth, 4 μ.

Taken in the plankton at the surface inside the kelp belt off San Diego in June. The structure of the lorica indicates a eupelagic distribution.

Tintinnopsis reflexa sp. nov.

Pl. XXVI. Fig. 2.

The lorica of this organism is cylindrical, finger-shaped, its length two and one-half times its diameter, with rounded fundus and reflexed oral rim. The sides are straight and at the mouth the wall is reflexed, forming a broadly rounded oral perimeter, and continues aborally parallel to and outside of the cylinder for one-tenth of its length, terminating in a smooth unmodified edge. The wall is thin, translucent and has the primary reticulations described by Biedermann ('93) and Brandt ('96) but

no secondary fenestration. The outer surface of the wall is sparsely strewn with numerous, small, irregular particles of a more highly refractive character than its own structure.

The animal has the form and structure usual in *Tintinnopsis*. There are two ellipsoidal nuclei centrally located and in the posterior end a single vacuole whose diameter at diastole equals half that of the lorica.

A reflexed oral margin is not found in any other species of *Tintinnidae*. The nearest approach to it appears in the flaring rims of such species as *Amphorella steenstrupi, A. acuta, Petalotricha ampulla, Tintinnopsis mortenseni, T. bütschlii,* and *T. campanula*. In none of these forms has this flaring rim much greater relative proportions than has the reflexed rim of *Tintinnopsis reflexa*. An exception to this limitation in extent appears to be presented in the problematical organism described by Cleve ('99) as *Fungella arctica* and referred by him to the *Tintinnidae*. The significance of this limitation in proportions lies, it seems, in the dependence of this projecting portion of the shell upon the *length of the cilia and intercalary cirri of the adoral ciliary plates*. In *T. reflexa* the distal edge of the lorica is located approximately at the line where the ends of the cirri of the adoral plates would fall when reflexed.

The general form of the lorica of this species approaches most nearly to that of *T. nitida,* described by Brandt ('96) from Karajak-Fjord in Greenland waters. It differs, however, from this species in the posterior reflexion of its more extended rim, in the minuteness and sparseness of the attached particles and in its smaller size.

Dimensions:—Length, 50 μ; diameter, 20 μ.

Taken in a vertical haul from 70 fathoms to surface off San Diego in July. The structure of the shell is indicative of a eupelagic distribution.

Tintinnopsis dadayi sp. nov.

Pl. XXVI. Figs. 3-5.

Lorica campanulate with expanded fundus, spreading margin and cylindrical central portion. Its length from apex to primary oral rim is 2 to 2.5 times its central diameter, 1.3 to 1.8

times that of the fundus and 1.1 to 1.35 times that of the oral margin. In some individuals the lorica is continued beyond the primary oral rim by a cylindrical extension whose diameter is the same as that of the body behind the oral rim as seen in Pl. XXVI, Figs. 4 and 5. A secondary oral rim may appear on the cylindrical extension. No trace of annulation was found in the lorica.

The wall of the lorica is formed by a single hyaline lamella to whose outer surface numerous highly refractive angular particles adhere.

This species is most nearly related to *T. bütschlii* Daday but differs from it in its smaller size, in the absence of annulations, in the more sharply differentiated and sometimes repeated oral rim and in the swollen fundus.

Dimensions.—Length, 80-108 μ; diameter of fundus, 55-65 μ, of the cylindrical part, 40-48 μ, of the oral rim 60-80 μ.

This species was taken frequently in the summer months in shoal waters near shore and evidently belongs to the coastal plankton.

Cyttarocylis quadridens sp. nov.

Pl. XXVII, Figs. 8-11. Pl. XXVIII, Fig. 18.

The lorica is elongated, vase-shaped, tapering abruptly one-third of the distance from the aboral end to a slender attenuately pointed pedicel which bears in its aboral half an expansion armed with four more or less salient tooth-like projections. The oral opening is about one-fifth of the total length in diameter, is squarely truncate, with a thick, very slightly flaring rim. From the mouth the body of the lorica tapers slightly to the sloping shoulders which contract to the slender sub-cylindrical pedicel whose greatest diameter is about one-sixth that of the mouth. The pedicel tapers gradually to about one-half its initial diameter and then spreads into a quadrangular skirt-like expansion which bears the four posteriorly spreading spines on its angles. The diagonal width is here about equal to the initial diameter of the pedicel. From the recessed posterior face of this expansion arises an attenuate terminal spine. The cavity of the lorica is constricted abruptly in the expanded

region of the pedicel and is continued as a slender tube nearly to the tip of the terminal spine.

The wall of the lorica is relatively thick, especially toward the oral margin where it measures 5 μ. It grows slightly thinner posteriorly especially in the expanded region of the pedicel and the terminal spine, where it measures only 2-3 μ in thickness.

The wall is composed of minute subregular prisms mainly hexagonal with occasional pentagonal or irregular ones, placed so that their ends form the inner and outer surfaces of the lorica. Their sides form the coarse subregular hexagonal meshwork which Brandt ('96) has designated as the secondary reticulum. The slightly rounded ends of the prisms form the whole, or at least a part, of the inner and outer lamellae of the wall. Under high magnification (Pl. XXVIII. Fig. 18) the outer lamella exhibits a very minute faint reticulation which Brandt has called the primary one. The diameter of the meshes of this primary reticulum is less than 1 μ, and that of the secondary about 5 μ. In the pedicel the secondary reticulum becomes indistinct and on the expansion and terminal spine it disappears altogether, apparently as a result of the greater thickness in the walls of the prisms.

Well preserved specimens of the inhabitant have not been observed within the lorica, though moribund individuals have been found there in a few instances.

This species varies considerably in the prominence and angle of divergence of the four salient spines on the pedicel and in the length of the terminal spine. The four spines are usually symmetrical with respect to each other but instances of asymmetry are occasionally seen (Pl. XXVII. Fig. 9). It belongs unquestionably to the form-cycle of *Cyttarocylis treforti*, described by Daday ('87) from Naples, which, however, has two lateral apophyses in place of a quadrangular expansion of the pedicel. Similar lateral apophyses also occur on the spirally striate form described by Cleve ('99*a*) as *C. hebe* var. *apophysata*. *C. treforti* occurs occasionally in the plankton of the Pacific off San Diego, but it does not appear to intergrade with the form here described as *C. quadridens*.

Observations on the method of formation of the lorica in *Cyttarocylis* are not to be found in literature and I have been unable to keep this species alive for prolonged examination in a microaquarium. It seems probable from the form of the lorica that this is built up from the terminal spine anteriorly, and that the quadrangular expansion on the pedicel with its four spines may in some way result from the presence of the four spiral lines of cilia on the body of the animal which pass from the adoral circlet toward the posterior end. They would form the natural lines of transit of substances gathered by the adoral circlet or extruded from the body and utilized in the formation of the lorica. The posterior ends of these lines of cilia may be regions where the shell-forming substances gather in the form of this quadrangular expansion with its more or less prominent spines. Anterior to this region the spiral course of the cilia and the greater freedom of movement on the part of the body of the animal would tend to facilitate the more regular distribution of the material and to bring about a transition from the quadrangular to the circular cross section of the shell.

Dimensions.—Total length, 430-450 μ; diameter of oral end, 90-100 μ; length of terminal spine, 35-50 μ; diagonal diameter at the expanded region of the pedicel, 12-18 μ.

This species is found generally, though rarely in large numbers, in the summer plankton of the Pacific off San Diego. It has been taken in vertical hauls from 185-35 fathoms to the surface very generally, and less frequently in surface catches. It appears to be a eupelagic species.

Cyttarocylis pulchra sp. nov.

Pl. XXVIII. Figs. 19-23.

This differs from the preceding in its proportions, in the possession of one to three rings about the anterior part of the lorica and in its very stout pedicel with a four-sided posterior portion. The lorica is vase-shaped, being cylindrical in its anterior third with a very slightly flaring mouth whose lip diminishes to a sharp edge. This section of the lorica bears one, or two, but more generally three external annulations which

result from a symmetrical increase of the wall to from 2 to 2.5 times its thickness in adjacent regions. The anterior ring is about one-fourth of the diameter of the mouth behind the rim, the second ring three-fourths, and the third a little less than five-fourths. The second and third are thus slightly nearer together than the first and second. The total length of the lorica is seven times its diameter between the rings and five times that on the rings.

The lorica tapers very gradually near its middle to the stout pedicel which with its terminal spine forms the posterior half of the total length. This pedicel is about one-third of the diameter of the anterior part measured between the rings, and changes in the posterior third of its length from a cylinder to a rectangular prism from whose flaring end arises the stout terminal spine. The four angles of the pedicel are carried out (on the skirt-like expansion) in projecting points like those of *C. quadridens* and in addition one similar point is intercalated on each margin of the overhanging ledge midway between the two corners of each face. The width of the faces is about one-fourth the diameter of the mouth of the lorica.

The cylindrical spine projects from the center of the recessed region at the base of the pedicel and ends in an acute tip. Its length is nearly one-half the diameter of the mouth, and its diameter less than one-fifth of its own length.

The cavity of the lorica conforms to the external contour with the exception that there are only very slight annular expansions beneath the rings, and that in the prismatic portion of the pedicel the lumen contracts suddenly to a slender canal which extends as a straight tube nearly to the end of the terminal spine.

The structure of the lorica is essentially similar to that of *C. quadridens.* It is composed of similar elements having a similar arrangement in all parts but the rings. In *C. quadridens* the wall is everywhere composed of a single layer of prisms but in *C. pulchra* the rings, as shown in Pl. XXVIII, Fig. 20, are formed by 2-3 layers of prismatic elements, which pass over into the single layer on either side. In the quadrangular sec-

tion of the pedicel the prisms which are thin-walled elsewhere become very thick-walled so that their central cavities are almost obliterated, giving a pitted appearance to the wall in this region. This wall is, as before stated, much thickened, but I have found only a single layer of prisms in it. It has a yellowish brown color which is in strong contrast with the hyaline character of the rest of the lorica. The presence of rings on the lorica of this species and the occurrence of loricae having only one or two rings raises an interesting question as to the method and significance of their formation. It seems probable that there occurs during the period of lorica formation a temporary suspension in the factors leading to its elongation without concurrent diminution in the supply of the materials from which the hexagonal prisms are formed, resulting in a local aggregation of the prisms in a ring. This process may, it seems, occur two or three times and at an approximately uniform interval. The structure in these particulars is probably correlated with some phase of activity of profound importance in the animal's economy which is subject to rhythmic repetition. Naturally the suggestion arises that division or possibly conjugation may afford the basis on which these features of shell structure rest. Observations on this point are lacking because of the great difficulty of keeping these most delicate pelagic organisms under laboratory conditions.

The animal has not been seen in a normal condition. Moribund individuals have three or more ellipsoidal nuclei.

Dimensions.—Total length, 405 μ; diameter of oral end, 70 μ; length of terminal spine, 35 μ; width of face of pedicel, 20 μ; diameter of rings 82 μ; thickness of wall, 6-8 μ; diameter of prisms, 2-4 μ.

This species has been found generally in the plankton of the Pacific off San Diego at all seasons of the year but more frequently in the summer. It is never very common and is more frequent in vertical catches than in those taken at the surface. It appears to be a eupelagic species.

Cyttarocylis torta sp. nov.

Pl. XXVII, Figs. 12-15. Pl. XXVIII, Figs. 16, 17.

This species has many points in common with the preceding. In proportions and form of the lorica, the relations of cylindrical portion and pedicel, and in the form of the expansion and terminal spine the two species are counterparts. *C. torta* differs from *C. pulchra*, however, in two prominent details of structure which have been constant in all of the numerous individuals of the species which have come under my observation. In the first place the annulation is not formed by 1-3 distinct rings as in *C. pulchra* but by a very broad thickened band whose anterior and posterior margins are somewhat enlarged, a condition which might arise by the thickening of the region between the first and second rings in *C. pulchra*. The anterior thickening is usually less prominent than the posterior and the intermediate belt is not uniformly or symmetrically thickened on all sides, thus presenting a variety of margins as the lorica is rolled about. A second narrowed ring is found in some individuals behind the broad band, and as in the two ridges in front of it, its anterior face is less abrupt than the posterior one, differing in this particular from the evenly rounded rings on *C. pulchra*.

The second structural feature differentiating this species from *C. pulchra* is the marked torsion of the quadrangular portion of the pedicel, which makes a turn of 90°-180° from right over to left (*cf.* Figs. 14 and 15). The torsion appears in the prominent lines which form the angles of this part of the pedicel and also in the several—usually three—fainter lines distributed on each face between the angles. These lines in common with those upon the angles, terminate in projecting points along the margin of the skirt-like expansion. There is some irregularity among different individuals in the number and distribution of these intermediate lines. The direction of the torsion is uniform in all loricae examined.

The finer structure of the lorica is essentially similar to that of *C. pulchra* as shown in the figures. The quadrangular portion of the pedicel is thick-walled occluding the lumen to a

slender tube which has, however, an ovoidal expansion just before it enters the terminal spine (Pl. XXVII, Fig. 12).

This species belongs to the form-cycle of *C. pulchra* to which species it is evidently closely related. The existence of two constantly present differential characters in the individuals of this species under my observation leads me, however, to regard it as distinct from *C. pulchra*. The nearest approach to intergrades appears in one individual of *C. pulchra* (Fig. 23) in which the second ring is slightly widened.

The formation of the twisted end of the pedicel in this species may be due to the rotation of the animal during the early period of shell formation. If so, the rotation must be in one direction constantly, or at least nearly so, during this period of formation. In locomotion the *Tintinnidae*, in common with other free-swimming ciliates, rotate about the long axis. I have not observed *C. pulchra* in activity, but in other species which I have seen in motion reversals in the direction of this rotation are not infrequent. It is difficult to find an explanation of the difference between the broad anterior band and the smaller posterior ring in *C. torta* on the supposition made in the case of the rings in *C. pulchra*, that they are attendant upon the repetition of some phase such as division or conjugation in the life history of the organism.

The structure of the lorica is similar to that of *C. pulchra* with the exception that there are 2-3, and sometimes as many as 5 layers of prismatic elements in the rings and collar and that the thickened region of the pedicel is relatively longer.

The animal has not been seen in normal condition.

Dimensions.—Total length, 450 μ; diameter of mouth, 65 μ, on rings, 90 μ; of pedicel, 18-25 μ; diagonal of pedicel expansion, 30 μ; thickness of wall, 2 to 4 μ; length of terminal spine, 40 μ.

This species has been taken sparingly in both summer and winter plankton of the Pacific at San Diego, but more abundantly in vertical than surface catches. It is apparently eupelagic in its distribution.

Cyttarocylis fasciata sp. nov.

Pl. XXVI. Figs. 6, 7.

Lorica elongated, subconical, its length five times its oral diameter. The posterior third contracts more rapidly than the anterior to a blunt, somewhat irregular, apex. The terminal third is curved slightly to one side so that the apex is asymmetrical. Near the mouth the lorica widens a little to a partially and irregularly everted lip.

The wall of the lorica is formed by a band of substance laid in a spiral of about 17 turns from right over to left (leiotropic) from the apex toward the mouth. The width of this band is not uniform; it varies from 0.2 to 0.6 of the oral diameter, being widest in the fourth and fifth turns from the apex, the region of most rapid diminution in calibre, and narrowing abruptly in the three apical turns, and more gradually toward the mouth. The band is placed somewhat obliquely to the trend of the side so that the posterior margin of each turn is set on the inner face of the anterior margin of the turn behind it (Pl. XXVI, Fig. 7). In the last turn at the oral end the width of the band diminishes gradually so that the mouth is squarely truncate.

The wall is composed of minute prismatic elements of very irregular form, with a varying number (3-6) of sides of irregular and unequal length. As with other species of *Cyttarocylis* here described, the ends of the prismatic elements form the inner and outer faces of the lorica. The irregularity of the pattern which they form in this species stands in strong contrast with the regular hexagonal type seen in species previously described in this paper.

The inhabitant of the lorica has not been observed.

This form belongs to that group of species of *Cyttarocylis* in which the material of the shell is laid down in bands as a result of intermittent activity of secretion or of spiral rotation or torsion of the body. Intermittent deposition yields the annulated type of lorica. When the process of extrusion of the prismatic elements or other lorica-forming substances is intermittent only during the latter part of shell formation, such loricae are produced as that of *C. annulata* of Ostenfeld and Schmidt ('01)

where the rings are limited to the anterior end. When intermittent deposition continues throughout the whole of shell formation, the entire lorica is composed of superposed rings of equal or unequal width as in *C. annulata* of Daday ('87) and *C. fistularis* [*Tintinnus fistularis* of Moebius('87)]. Jörgensen is probably correct in regarding the latter species as identical with *C. helix* (Clap. et Lach.) Jörg. in which the structure of the lorica is imperfectly known, but appears from the figure of Claparède and Lachmann ('58-'59) and the discussion of Jörgensen ('99) to consist of an apical portion, which is formed by a broad band spirally wound, and a superposed oral portion made up of a number of narrower transverse rings.

When the deposition of shell material is continuous and attended by torsion we may have the spiral type of banded lorica in the anterior end as in *C. claparedi* of Daday ('87) and the nearly related if not identical *C. ehrenbergi* var. *subannulata* of Jörgensen ('99), or throughout the whole lorica as in *C. pseudannulata* of Jörgensen ('00) and in the species here described.

The type of shell structure in *C. fasciata* suggests the slow rotation of the animal in a constant direction during the deposition of the shell-forming substance (from which the prismatic elements are formed) and the localization and limitation of the region of its extrusion to a single place upon the animal. It seems desirable that all annulate forms of the *Tintinnidae* should be reinspected carefully for spiral structure.

It is evident that the spiral structure of the shell is of great importance in assisting in the rotation of this structure during active locomotion of the animal and maintaining it during passive movement through the water, as for example during its sinking, and that with the rotation there comes a corresponding increase in the molecular friction and that the flotation of the organism is thus facilitated.

This species is most nearly related to *C. helix* (Clap. et Lach.) Jörg., from which it differs in its much greater size (length 520μ to 150-200μ in *C. helix*), and in the greater width of the anterior bands which are also plainly spiral, while in *C. helix* they are probably transverse and are very narrow. The proportions of the two species are also different. *C. fasciata* is conical,

while *C. helix* is cylindrical with more or less pronounced curvature of the tapering apex.

Dimensions—length, 520μ; diameter of mouth, 100μ; at apex, 20μ; width of spiral band, 20-60μ.

This species was taken but once, in a vertical haul from 35 fathoms to surface, 8 miles off Pt. Loma in June.

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EXPLANATION OF PLATE XXVI.

Fig. 1.—Lateral view of lorica of *Tintinnus serratus*, ×615.

Fig. 2.—Lateral view of lorica of *Tintinnopsis reflexa*, ×600.

Fig. 3.—Lateral view of lorica of *Tintinnopsis dadayi*, ×375. Individual with primary oral rim only.

Fig. 4.—The same of a second lorica, showing both primary and secondary oral rims, ×190.

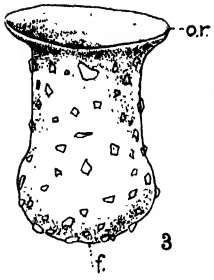
Fig. 5.—The same of a third lorica, in which the secondary oral rim is only partially developed, ×375.

Fig. 6.—Lateral view of lorica of *Cyttarocylis fasciata*, ×490.

Fig. 7.—Longitudinal optical section through wall of lorica of *C. fasciata*, ×1225.

ABBREVIATIONS.

ab. ap.—aboral aperture.
f.—fundus.
o. ap.—oral aperture.
o. r.—oral rim.
p. o. r.—primary oral rim.
pr. el.—prismatic elements.
r. m.—reflexed margin.
s. o. r.—secondary oral rim.
sp. b.—spiral band.



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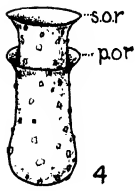

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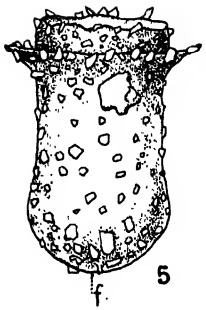

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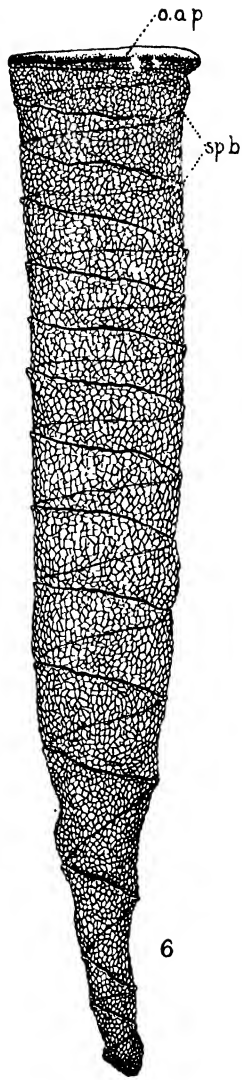

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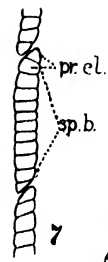

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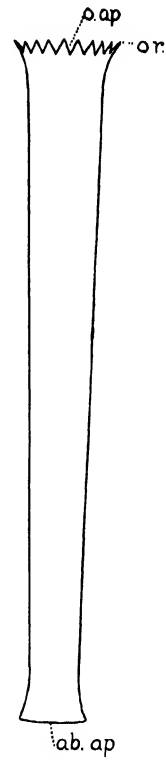

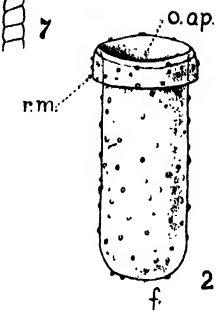

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EXPLANATION OF PLATE XXVII.

Fig. 8.—Lateral view of lorica of *Cyttarocylis quadridens*, ×250.

Fig. 9.—Lateral view of posterior ends of lorica of *C. quadridens*, showing asymmetry and degrees in development of the lateral spines, ×250.

Fig. 10.—Lateral view of posterior end of lorica of *C. quadridens*, showing lumen, ×1200.

Fig. 11.—Optical section of wall of lorica of *C. quadridens*, showing prismatic elements, ×1200.

Fig. 12.—Optical section through posterior end of lorica of *Cyttarocylis torta*, showing lumen, ×600.

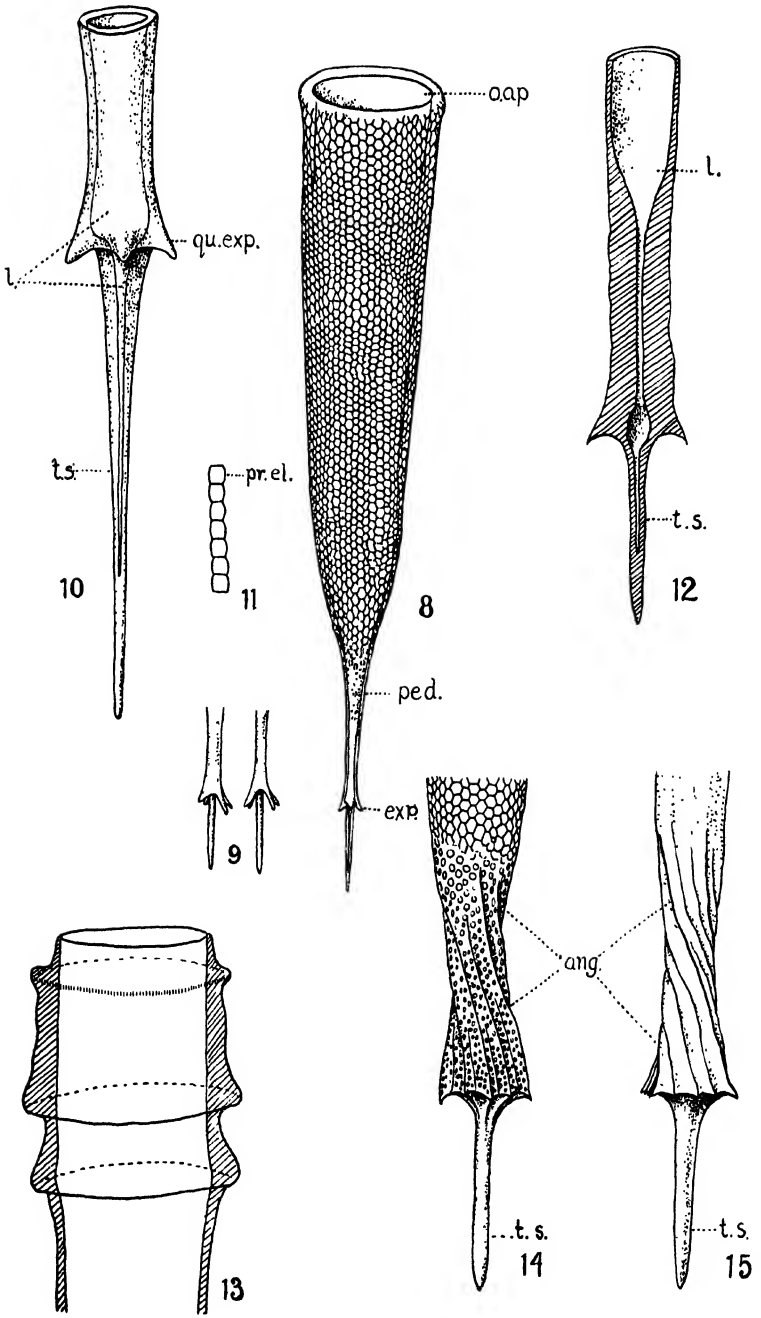
Fig. 13.—Anterior end of lorica of *C. torta*, viewed as a transparency. Lorica with additional posterior ring, ×320.

Fig. 14.—Posterior end of lorica of *C. torta*, showing 90° of torsion, ×600.

Fig. 15.—Another lorica of the same, showing 180°, ×600.

ABBREVIATIONS.

ang.—angles of quadrangular pedicel.
exp.—expansion of pedicel.
l.—lumen of lorica.
o. ap.—oral aperture.
ped.—pedicel.
pr. el.—prismatic elements.
qu. exp.—quadrangular expansion of lorica.
t. s.—terminal spine.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EXPLANATION OF PLATE XXVIII.

Fig. 16.—Lateral view of lorica of *Cyttarocylis torta*, having no additional ring, ×250.

Fig. 17.—Optical section and inner surface of anterior end of lorica of *C. torta*, showing prismatic structure, ×375.

Fig. 18.—Surface of lorica of *C. quadridens*, showing primary and secondary reticulations, ×1100.

Fig. 19.—Lateral view of lorica of *Cyttarocylis pulchra*, having three rings, ×250.

Fig. 20.—Optical section and inner surface of lorica of *C. pulchra*, showing prismatic structure, ×500.

Fig. 21.—Posterior end of lorica of *C. pulchra*, ×500.

Fig. 22.—Optical section of same, showing lumen, ×500.

Fig. 23.—Anterior end of lorica of *C. pulchra*, viewed as a transparency. Lorica with modified central ring, ×250.

ABBREVIATIONS.

o. ap.—oral aperture.
ped.—pedicel.
pr. el.—prismatic elements.
pr. ret.—primary reticulation.
qu. ex.—quadrangular expansion.
r.—rings.
t. s.—terminal spine.
sec. ret.—secondary reticulation.

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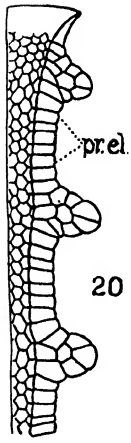

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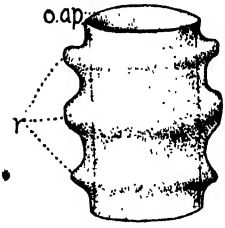

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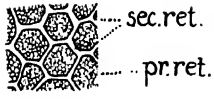

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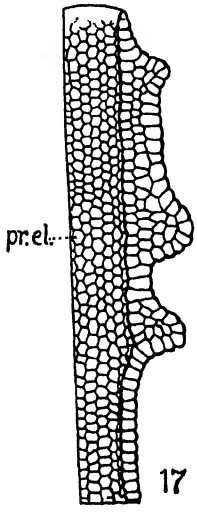

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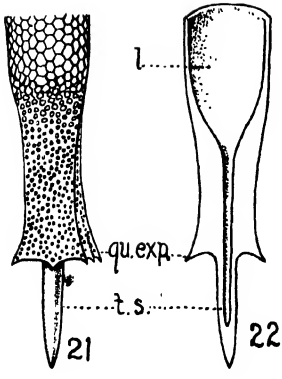

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